Stabilization of Polyethylene Glycol in Archaeological Wood

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Stabilization of polyethylene glycol in archaeological wood

Ph.D. Dissertation

By

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2009

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Cover Photo: The Vasa ship in Stockholm photographed by Hans Hammarskiöld.

Preface

This thesis describes results obtained during my Ph.D., which was supervised by Professor Søren Hvilsted of the Department of Chemical and Biochemical Engineering at the Danish Technical University (DTU). Senior Scientist Jens Glastrup in the Department of Conservation at the Danish National Museum was co-supervisor.

The project involved a rather large number of institutions, to various extents. These institutions are the National Maritime Museums of Sweden, The Danish Research School of Cultural Heritage, the Department of Conservation at the Danish National Museum, the Department of Chemical and Biochemical Engineering at the Danish Technical University (DTU), the Biosystems Division and the Danish Polymer Centre both at Risø DTU National Laboratory for Sustainable Energy. The experimental work was mainly carried out at the laboratories of the Department of Conservation at the Danish National Museum and at the named departments at Risø.

The project was co-financed by the Department of Conservation at the Danish National Museum, the Danish Ministry of Culture and by the National Maritime Museums of Sweden research project "Save the VASA" sponsored by The Bank of Sweden Tercentenary Foundation, The Swedish National Heritage Board, The Swedish Foundation for Strategic Research (SSF), The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), and The Swedish Agency for Innovation Systems (Vinnova).

The thesis is partly based on journal publications and partly on self-contained chapters. It consists of three main chapters. The first chapter gives results that supplement the first article, which is reprinted in Appendix 1. The article is:

Mortensen, M.N., Egsgaard, H., Hvilsted, S., Shashoua, Y., Glastrup, J., Characterisation of the polyethylene glycol impregnation of the Swedish warship Vasa and one of the Danish Skuldelev Viking ships, Journal of Archaeological Science 34 (2007) 1211-1218.

In this work MNM participated in the planning of the experiments, prepared the samples, performed extractions and prepared extracts for analysis by MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry) and SEC (Size Exclusion Chromatography) which was then performed by technicians at Risø. MNM wrote the manuscript.

The second chapter describes results that supplement a finished manuscript that has not yet been submitted. It is the idea to submit the manuscript to Journal of Archaeological Science, it is printed in Appendix 2. The manuscript is:

Mortensen, M.N., Egsgaard, H., Hvilsted, S., Shashoua, Y., Glastrup, J., Stability of polyethylene glycol in conserved wooden shipwrecks – the effect of matrix. Intended for Journal of Archaeological Science.

In this work MNM participated in the planning of the experiments, conducted the experiments and wrote the manuscript.

The third chapter mostly deals with unpublished work. This will probably not get published until more experiments have been carried out to support it. The result that was in fact published was described thoroughly in the chapter. This way reading the article is not required in order to

understand the chapter. The published results are in the following article, which is printed in Appendix 3:

Glastrup, J., Shashoua, Y., Egsgaard, H., Mortensen, M.N., Formic and acetic acids in archaeological wood. A comparison between the Vasa Warship, the Bremen Cog, the Oberländer Boat and the Danish Viking Ships, Holzforschung 60 (2006) 259-264.

MNM contributed to this work by participating in the design of the analyses.

Brede, December 1st 2008

Martin N. Mortensen

Contents

Preface i
Contents iii
Abstract v
Resumé vi
Abbreviations vii

1 Background 1
1.1 The warship Vasa and the Skuldelev Viking ships *1*
1.2 Wood anatomy *2*
1.3 Chemistry of the major wood constituents *3*
1.3.1 Cellulose *3*
1.3.2 Hemicellulose *3*
1.3.3 Lignin *3*
1.4 Microbial degradation of wood *5*
1.5 Conservation of waterlogged wood *5*
1.5.1 The alum method *6*
1.5.2 Polyethylene glycol impregnation *7*
1.5.3 Freeze-drying *7*

2 PEG state and distribution in the Vasa and the Skuldelev ships 9
2.1 Further experimental *11*
2.2 Further results and discussion *11*
2.3 Further conclusions *17*

3 Accelerated ageing of PEG and TEG - the effect of matrix 19
3.1 Polyethylene glycol degradation *19*
3.1.1 Thermo-oxidative degradation *19*
3.1.2 Radiation induced degradation *23*
3.1.3 Pyrolytic degradation *24*
3.1.4 Stabilisation of PEG *25*
3.2 Further experimental *27*
3.3 Further results and discussion *28*
3.3.1 Accelerated ageing of TEG *28*
3.3.2 Accelerated ageing of PEG 1500 *32*
3.3.3 Effect of additives on rate of degradation of TEG *32*
3.4 Mechanism *35*
3.4.1 PEG degradation mechanisms *35*
3.4.2 Antioxidant mechanism *39*
3.4.3 Relation to PEG-treated wood *41*
3.5 Further conclusions *43*

4 Formic acid as a marker for PEG degradation **45**
4.1 Method for measuring formic acid concentration ***47***
4.1.1 Experimental *47*
4.1.2 Results and discussion *48*
4.1.3 Conclusion *49*
4.2 Method for determining $H^{12}COO^-/D^{13}COO^-$ and $^{12/13}CO_2$ ratios ***50***
4.2.1 Experimental *50*
4.2.2 Results and discussion *51*
4.2.3 Conclusions *55*
4.3 Method for isolating formic acid from wood ***56***
4.3.1 Experimental *56*
4.3.1.1 Extraction 56
4.3.1.2 Vacuum distillation 56
4.3.1.3 Ion exchange 57
4.3.1.4 Second vacuum distillation 58
4.3.1.5 Oxidation 58
4.3.1.6 Sample pre-treatment and AMS 60
4.3.2 Results and discussion *61*
4.3.3 Conclusions *63*
4.4 Formic acid content in PEG-treated wooden shipwrecks ***64***
4.4.1 Experimental *64*
4.4.2 Results and discussion *64*
4.4.3 Conclusions *67*
4.5 Radiocarbon analysis of formic acid in the Vasa ***68***
4.5.1 Experimental 69
4.5.2 Results and discussion 69
4.5.3 Conclusions 73
4.6 Conclusions ***73***

5 Summary **75**
5.1.1 Outlook 76
5.1.2 Overall conclusion 77

References **78**

Appendix 1 **86**
Appendix 2 **95**
Appendix 3 **114**
Appendix 4 **121**
Appendix 5 **122**

Abstract

Polyethylene glycol (PEG) has often been used to impregnate waterlogged archaeological wooden objects. Examples of PEG-impregnated shipwrecks include the Vasa in Sweden, the Skuldelev Viking Ships in Denmark, the Oberländer boat in Austria and the Bremen cog in Germany. The present study deals with PEG stability and degradation in the timbers of conserved shipwrecks.

PEG impregnation of the Vasa and the Skuldelev Viking ships was characterised by recording Attenuated Total Reflectance Fourier Transform-InfraRed (ATR FT-IR) spectra and by extracting PEG at various depths. This showed that a large amount of PEG is in the surface layers of the wood and smaller amounts of PEG are found deeper inside the wood. For PEG extracts from the Vasa, Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) showed that non-degraded wood is too dense for PEG 4000 to migrate very deeply. PEG 1500 and PEG 600 are found at all depths. PEG with a tailing molecular weight distribution (molecular weight < 2000 g/mol) was detected in a sample from the Skuldelev Viking ships with MALDI-TOF MS and Size Exclusion Chromatography (SEC). Such tailing was also observed in a batch of PEG that was left over from the conservation of the Vasa, using MALDI-TOF MS. This observation makes it problematic to use tailing in conserved wood as a sign of PEG degradation. Tailing was also observed in a sample from the Skuldelev Viking ships, implying that degradation could have taken place but the tailing cannot be used to prove it. Some of the MALDI-TOF MS spectra of PEG from the Vasa and the Skuldelev Viking ships had small “satellite peaks” at 14, 16, 26 and 42 mass units over the parent PEG ion. PEG sodium chloride salt clusters, overly intense ionisation and potassium ion contamination are the most plausible explanations. Thus satellite peaks are artefacts of the MALDI-TOF MS analysis of PEG extracts.

Accelerated ageing of the PEG model molecule tetraethylene glycol (TEG), was carried out at 70 °C with air or nitrogen bubbling through. Gas Chromatography-Mass Spectrometry (GC-MS) and weighing of the TEG was used to monitor the degradation. It showed that oxidation dominated the degradation at 70 °C and that mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol were detected along with formic acid as products of the degradation. Accelerated ageing of PEG 1500 was carried out at 90°C under air using ElectroSpray Ionization Mass Spectrometry (ESI MS) to analyse the products. Satellite peaks appeared at 28 and 14 mass units above the parent PEG ion during ageing. The peaks were assigned to PEG formates and tentatively to in-chain esters, respectively. TEG thermo-oxidation was inhibited by several different components added in small quantities. These include KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2, $CuSO_4$, fresh oak wood sawdust and a scraping from the Vasa that contained gypsum. This suggests that PEG in a wood matrix could be less prone to degradation than might be anticipated.

A method was developed for Solid Phase Micro Extraction Gas Chromatography-Mass Spectrometry (SPME GC-MS) to measure formic acid concentrations in PEG impregnated objects. The average concentrations in the Vasa, the Oberländer boat, the Skuldelev Viking ships and the Bremen cog were between 0.012 % and 0.063 % by weight. Translation of these contents into PEG degradation since the onset of conservation suggests that it would take approximately 70000 years to completely degrade all the PEG that is estimated to be in the Vasa or the Skuldelev ships, assuming that two formic acid molecules are produced for every ethylene glycol monomeric unit degraded in the PEG. A method to isolate formic acid from PEG-impregnated archaeological wood for subsequent ^{14}C-analyses by Accelerator Mass Spectrometry (AMS), was successfully validated. It was found that formic acid in a sample from the Vasa was between 100 and 88 percent petrochemical (PP) where 0 PP corresponds to the Vasa wood and 100 PP corresponds to PEG. This demonstrates that the formic acid is mainly a product of PEG degradation and not of wood. This confirms that at least some PEG degradation has taken place at some point since the onset of conservation of the Vasa sample.

Resumé

Polyethylen glycol (PEG) er ofte blevet anvendt til imprægnering af vanddrukne arkæologiske træ genstande. Vasa i Sverige, Skuldelev skibene i Danmark, Oberländer båden i Østrig og Bremer koggen i Tyskland er eksempler på PEG imprægnerede skibsvrag. Nærværende arbejde vedrører PEG stabilitet og nedbrydning i træ fra konserverede skibsvrag.

Vasa og Skuldelev skibenes PEG imprægnering blev karakteriseret ved ATR FT-IR (Attenuated Total Reflectance Fourier Transform-InfraRed) spektroskopi og ekstraktion af PEG i forskellige dybder. Dette viste at der var meget PEG i overfladen og mindre PEG dybere inde i træet. MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry) viste at ikke-nedbrudt træ er for tæt til at PEG 4000 kan trænge særlig dybt ind i det, mens PEG 1500 og PEG 600 findes i alle dybder. Tailing blev observeret (molekylvægt <2000 g/mol) i molekylvægtsfordelingen for PEG i en prøve fra Skuldelev skibene ved hjælp af MALDI-TOF MS og SEC (Size Exclusion Chromatography). Tailing blev også observeret med MALDI-TOF MS, i en portion PEG, som var tilovers efter konserveringen af Vasa. Herved bliver anvendelsen af tailing som tegn på PEG nedbrydning i konserveret arkæologisk træ, problematisk. Tailing blev observeret i en prøve fra Skuldelev skibene, hvilket antyder, at nedbrydning kunne have fundet sted, tailing kan imidlertid ikke bruges som bevis herfor. Nogle af MALDI-TOF MS spektrene af PEG fra Vasa og Skuldelev skibene havde små "satellit toppe" ved 14, 16, 26 og 42 masse enheder, over PEG moder ionen. PEG-natriumchlorid saltklynger, for intens ionisering samt kalium ion kontaminering er de mest sandsynlige forklaringer. Satellit toppene anses således for artefakter frembragt ved MALDI-TOF MS analysen af PEG ekstrakterne.

PEG model molekylet, tetraethylen glycol (TEG), blev udsat for accelereret ældning ved 70 °C med luft eller kvælstof gennembobling. Processen blev fulgt ved vejning og med GC-MS (Gas Chromatography-Mass Spektrometry). Dette viste at oxidation dominerer nedbrydningen ved 70 °C, at mono-, di- og triethylen glycol, mono- og diformiaterne af mono-, di-, tri- og tetraethylen glycol samt myresyre, var nedbrydningsprodukter. PEG 1500 blev udsat for accelereret ældning ved 90°C under luft, idet produkterne blev analyseret med ESI MS (ElectroSpray Ionization Mass Spectrometry). Satellit toppe voksede frem ved 28 og 14 masse enheder over PEG moderionen i løbet af ældningen. Førstnævnte satellit top blev tilordnet til PEG formater, toppene ved 14 blev tilordnet tentativt til in-chain estre. Flere forskellige komponenter, tilsat i små mængder, hæmmede thermo-oxidation af TEG. Disse indbefatter KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2, $CuSO_4$, savsmuld af egetræ og afskrabninger fra Vasa som også indeholder gips. Dette antyder at PEG i en træ matrix er mindre udsat for nedbrydning end antaget hidtil.

En metode blev udviklet med SPME GC-MS (Solid Phase Micro Extraction Gas Chromatography-Mass Spectrometry), til at måle myresyre koncentrationer i PEG imprægnerede genstande. Middel koncentrationerne i Vasa, Oberländer båden, Skuldelev skibene og Bremer koggen lå mellem 0.012 % og 0.063 % (vægtprocent). Omsættes disse koncentrationer til PEG nedbrydning fra konserveringen blev påbegyndt, ville det tage omtrent 70000 år at nedbryde alt det PEG som anslås at være i Vasa og Skuldelev skibene, forudsat at to myresyre molekyler dannes for hver ethylenglycol monomer der nedbrydes. En metode blev udviklet, til at isolere myresyre fra PEG imprægnerede arkæologiske træ genstande med henblik på efterfølgende ^{14}C-analyse ved AMS (Accelerator Masse Spektrometri). Det viste sig at myresyre isoleret fra en Vasa prøve, var mellem 100 og 88 Procent Petrokemisk (PP) hvor 0 PP svarer til Vasa træ og 100 PP svarer til PEG. Dette viser at myresyren hovedsageligt er dannet ved PEG nedbrydning, hvilket bekræfter at PEG nedbrydning har fundet sted efter konserveringen af Vasa blev påbegyndt, om end i begrænset omfang.

Abbreviations

A_{ABS}	Absolute Activity of sample
AMS	Accelerator Mass Spectrometry
API	Atmospheric Pressure Inlet
A_S	Activity of sample
A_{SN}	Activity of sample normalised
A_O	Activity of oxalic acid standard
A_{ON}	Activity of oxalic acid standard normalised
ATR FT-IR	Attenuated Total Reflectance Fourier Transform-InfraRed
BHT	Butylated HydroxyToluene
BP	Before Present
CID	Collision Induced Dissociation
DP	Degree of Polymerization
DSC	Differential Scanning Calorimetry
E°	Standard reduction potential
EG	Ethylene Glycol
EI	Electron Impact
ESI MS	ElectroSpray Ionization Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Pressure Liquid Chromatography
ID	Inner Diameter
L	Length
LP	Laser Power
LV SEM	Low Vacuum SEM
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry
NMR	Nuclear Magnetic Resonance
OD	Outer Diameter
PDB	PeeDee Belemnite
PDMS	PolyDiMethyl Siloxane
PEG	PolyEthylene Glycol
PEO	PolyEthylene Oxide
Photo-DSC	Photo - Differential Scanning Calorimetry
PmC	Percent modern Carbon
PP	Percent Petrochemical
PSA	Primary Secondary Amine
RCA	RadioCarbon Age
RCS	Random Chain Scission
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SHE	Standard Hydrogen Electrode
SIS	Selective Ion Storage
SPME	Solid Phase Micro Extraction
TEG	TetraEthylene Glycol
TG	ThermoGravimetry
THF	TetraHydroFurane
TIC	Total Ion Count
TLC	Thin Layer Chromatography

1 Background

1.1 The warship Vasa and the Skuldelev Viking ships[1,2]

Several museums around the world display old wooden shipwrecks as the main attraction. A few examples include; the warship Vasa in Stockholm (SE), the Bremen cog in Bremerhaven (D), the Batavia in Freemantle (AU), the Skuldelev Viking ships in Roskilde (DK), the Hjortspring boat in Copenhagen (DK) and the Mary Rose in Portsmouth (UK), the latter is under conservation but still accessible to visitors.

These ships have been sitting on the bottom of the sea, of a riverbed, in the soil or sediment for hundreds of years. There were differences in the state of preservation when they were discovered. In some cases, for example the Vasa, a regular salvage was sufficiently gentle. In other cases, such as the Skuldelev ships, underwater excavation or excavation in a cofferdam was necessary.

The Vasa sank in 1628 less than a mile into its maiden voyage from Stockholm. The ship was constructed as the largest and most heavily armed warship at its time. It had much too heavy cannons on the two gun-decks, and too little ballast. As a result the ship capsized in a gust of wind, and sank shortly after. The ship was well preserved when it was salvaged in 1961 after 333 years on the seabed. Conservation was initiated in 1962 and finished in 1989, then a new museum fully dedicated to the Vasa, was opened.

Figure 1. Left: the Vasa in Stockholm (photo: Hans Hammarskiöld). Right: Skuldelev wreck number 1 in Roskilde.

The five Skuldelev Viking ships were sunk on purpose some time during 1070-1090 AD, near the present town Skuldelev, to block the narrow channel “Peberrenden” in the Roskilde fjord. The site was excavated between 1957 and 1962. This was done as an underwater excavation from 1957 to 1959, and finished in a cofferdam in 1962. This allowed better access to the fragile remains that had settled flat in the sand as individual parts but in arrangements representing each of the five ships. The parts were conserved individually, and then reassembled inside the museum between 1968 and 1969. The Viking Ship Museum in Roskilde was officially opened in 1968.

1.2 Wood anatomy[3,4]

Many waterlogged archaeological wooden objects, especially boats, are made from oak. Oak is a hardwood (Angiosperm) characterised by good mechanical properties compared to softwoods (Gymnosperms) such as pine or spruce. The anatomy of hardwood is illustrated in Figure 2. Figure 2 (left) shows a Scanning Electron Microscopy (SEM) picture of oak. It is seen that the wood is composed of long thin closely packed cells. Tracheids, vessels and rays are the most abundant cell type in the picture. The tracheids and the vessels are oriented axially (parallel to the stem of the tree), the rays run in the radial direction (between the centre and the bark).

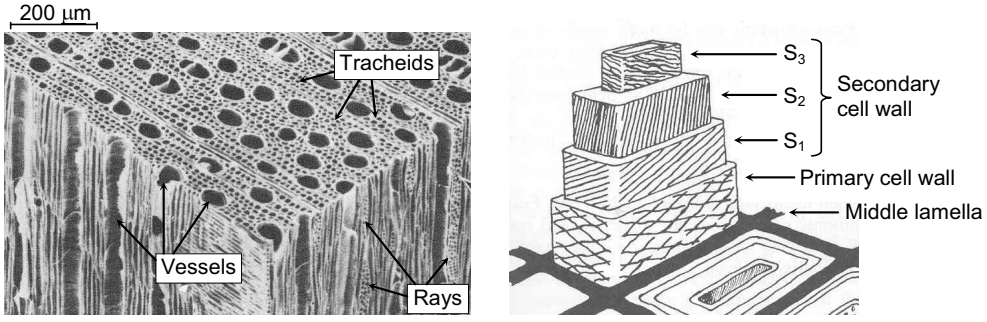


Figure 2. Left: Scanning Electron Micrograph (SEM) of fresh oak. The wood has been cut in such a way that a corner points out of the picture. The plane at the top of the picture is the cross section of the wood, the plane to the left is the radial plane and the plane to the right is the tangential plane. Three cell types are abundant; tracheids, vessels and rays (modified after Harada *et al.*[5]). Right: Illustration of the cell wall in a tracheid. The cells are held together by the middle lamella, the cell wall is divided into a primary and a seconday layer, the secondary cell wall is subdivided into three layers called S_1, S_2 and S_3. The layers are defined by the micro fibril orientations (modified after Björdal[6]).

The tracheids are packed closely together and held in place by the lignin rich middle lamella (Figure 2 right). The cell wall is rich in cellulose microfibrils, but also contains lignin and hemicellulose. It is divided into four distinct layers defined by the cellulose microfibril orientation. In the primary cell wall the cellulose microfibrils are loosely oriented in a web-like structure. The secondary cell wall consists of three subsections known as the S_1, S_2 and the S_3 layers. The microfibrils in S_1 wind around the cell at a large angle to the longitudinal axis. In S_2, which is the thickest layer, the microfibrils wind around the cell at a small angle to the longitudinal axis. In S_3, microfibrils wind around the cell at a large angle to the longitudinal axis, but in the opposite direction than in S_1 and S_2. The lignin in the cell wall incrusts the cellulose microfibrils, hemicellulose is often located between the microfibrils and between microfibrils and the lignin where it is covalently bound. Cellulose microfibril- and lignin contents reflect the physical properties needed in a particular part of the tree. High lignin contents are found in the parts of a tree that need compression strength and a high content of cellulose microfibrils is found where the tree needs tensile strength.

1.3 Chemistry of the major wood constituents[4]

1.3.1 Cellulose

Cellulose is a semi-crystalline polysaccharide that constitutes approximately half the dry weight of wood. It is a linear polymer consisting of D-glucose units linked by β-1,4-glucosidic bonds (Figure 3). The degree of polymerisation (DP) for wood cellulose is around 10000 D-glucose units.

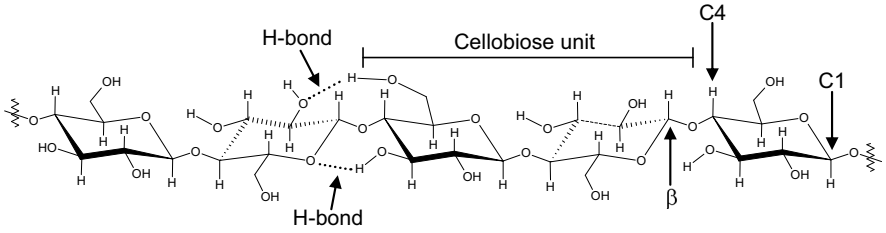


Figure 3. Section of a cellulose polymer chain consisting of D-glucose units linked by β-1,4-glucosidic bonds. Every second D-glucose unit is rotated 180°, two D-glucose units in such a conformation are known as a cellobiose unit, the structural repeating unit of cellulose. The D-glucose units are hydrogen bonded intramolecularly as indicated, and intermolecularly (not shown).

It is seen from the illustration that every second glucose unit is rotated approximately 180°. Two such D-glucose units, where one is rotated 180° relative to the other, constitute a cellobiose unit, which is the structural repeating unit of cellulose. Cellulose is extensively hydrogen bonded. As indicated in Figure 3, intramolecular hydrogen bonding is observed between the ring oxygen atom and the C3 hydroxy group and between the C2 and C6 hydroxy groups, on adjacent cellulose units. Intermolecular hydrogen bonding is also observed, this too involves ring oxygen and hydroxy groups.

1.3.2 Hemicellulose

Wood contains 20-30% (dry weight) noncellulosic polysaccharides. Hemicellulose is an important part of this fraction. It is a branched heteropolymer containing some or all of the carbohydrates: D-xylose, L-arabinose, L-rhamnose, D-glucose, D-mannose, D-galactose, and 4-o-methyl glucuronic acid. Sometimes the carbohydrates are acetylated. Hemicellulose is amorphous and the DP is low compared to that of cellulose. Covalent bonds are sometimes present between hemicellulose and lignin (see Figure 5), the resulting molecules are termed lignin-carbohydrate complexes (LCC). In hardwoods D-xylose residues are linked to lignin by ether bonds and glucuronic acid residues by ester bonds.

1.3.3 Lignin

Lignin is an aromatic polymer that constitutes 20-30% (dry weight) of wood. It is a polymer of three different monomers known as monolignols; p-coumaryl alcohol, coniferyl alcohol and sinapylalcohol (Figure 4).

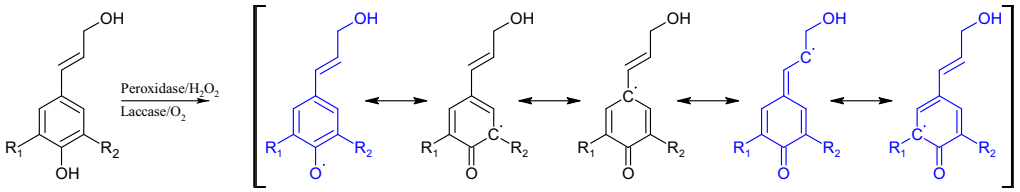


Figure 4. The three common monolignols p-Coumaryl alcohol (R_1=R_2=H), coniferyl alcohol (R_1=H, R_2=OCH_3) and sinapyl alcohol (R_1=R_2=OCH_3) are dehydrogenated by either peroxidase and H_2O_2, or by laccase and O_2 to form radicals that polymerise.

The bio-polymerisation starts by hydrogen atom abstraction from the phenol group of the monolignol (dehydrogenation) by the enzyme peroxidase and H_2O_2 or by laccase and O_2 to produce the corresponding phenoxyradical. The radicals then combine to form quinine methides that again undergo a series of reactions to form dilignols. The dilignols are dehydrogenated again and combine with other di- or monolignol radicals to form the lignin polymer. In softwood lignin coniferyl alcohol is the most abundant monomer. For steric reasons only three of the five possible resonance forms of this monolignol combine to form dilignols (blue colour in Figure 4).

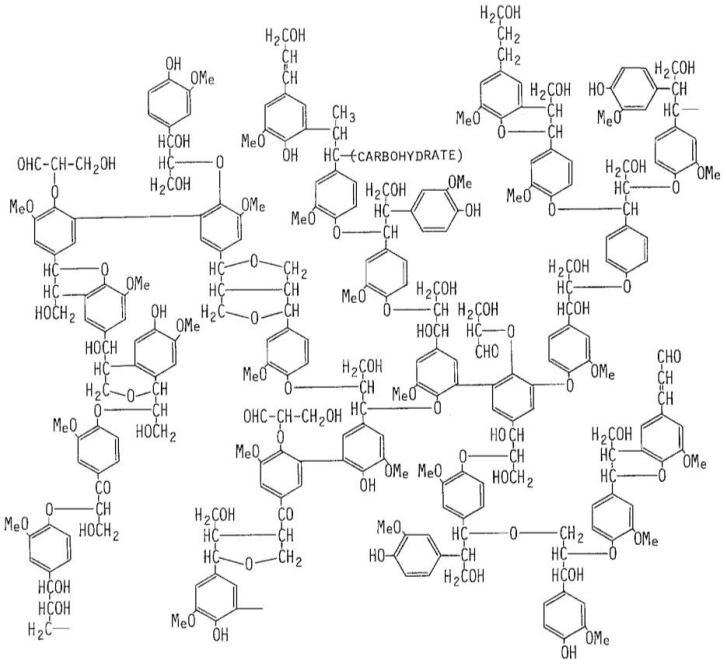


Figure 5. A structural model of a softwood lignin from Sakakibara.[7]

The monolignol radicals combine in many different ways, and some plants polymerise a mixture of the three different monolignols. This gives lignin a very complex structure, which is illustrated in Figure 5.

1.4 Microbial degradation of wood[6]

A range of micro-organisms is able to use wood as an energy source thereby degrading the material. Two groups of fungi, known as brown rot and white rot (both belonging to the basidiomycete group), degrade wood at a high rate. They are unable to live in a marine environment, though, because the amount of oxygen dissolved in seawater is too low. As a consequence, traces of brown rot or white rot on a waterlogged archaeological object is evidence that the rotting has taken place before burial (when the object was in its original use). Another group of fungi known as soft rot (belonging to the ascomycete and fungi imperfecti groups) is more common on wood in soil and water because they thrive at the lower oxygen levels found here. Soft rot does not degrade wood as fast as brown rot and white rot. Soft rot in waterlogged archaeological wood is mostly found in the S_2 layer of the cell wall where it degrades the cellulose microfibrils. The hyphae form channels in which they grow, following the S_2-microfibrillar orientation. This can be observed in a polarised light microscope as shown in Figure 6 left.

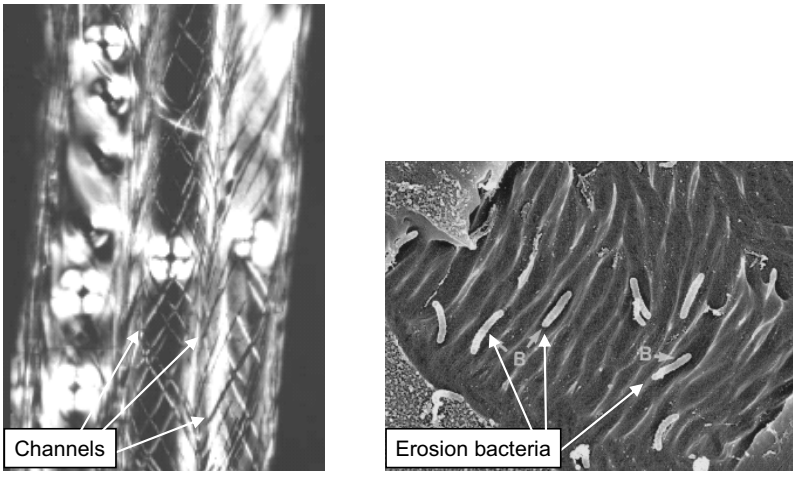


Figure 6. Left: Polarised light micrograph showing a longitudinal section of archaeological pine tracheids attacked by soft rot. The soft rot form channels, primarily in the S_2 layer, with the characteristic S_2-microfibril angle (compare to Figure 2 right). Picture from Björdal *et al.*[8] Right: Scanning Electron Micrograph (SEM) showing erosion bacteria within their troughs in an archaeological pine pole. Picture taken from Björdal *et al.*[9]

Besides fungi, three groups of bacteria are known to degrade wood. They do so under near-anaerobic conditions at a low rate. The bacteria are grouped on the basis of their specific micro-morphological features of attack; tunnelling, cavitation and erosion (Figure 6 right). Like soft rot, the bacteria are mostly found in the S_2 layer of the cell wall. They are the main cause of degradation of waterlogged wood in sediment or in oxygen-depleted (over-fertilised) water.

1.5 Conservation of waterlogged wood

If waterlogged wood is dried without special precautions it shrinks, sometimes up to 70%.[10] This is illustrated in Figure 7 (left) where two pieces of the same waterlogged ash pole (same initial diameter) are shown. The thin one was dried without conservation, the thick one received proper conservation. The phenomenon is shown on a microscopic scale in Figure 7 (middle and right). LV-

SEM (Low Vacuum Scanning Electron Microscope) pictures show collapsed (middle) and intact (right) ash wood. The intact wood was freeze dried (explained later), an intact vessel and some tracheids are clearly distinguished in the conserved wood. In the untreated wood, all the cells have collapsed.

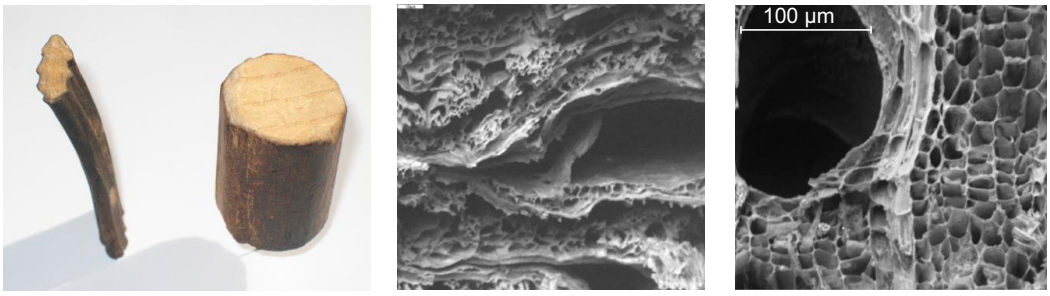


Figure 7. Left: Two pieces of the same waterlogged ash pole (same initial diameter), one dried without conservation (the thin piece), the other conserved using the freeze drying technique (the thick piece). Middle: LV-SEM picture of waterlogged ash wood that has been dried without conservation. Right: LV-SEM picture of conserved waterlogged ash wood. LV-SEM recorded by Ulrich Schnell at the School of Conservation in Copenhagen and by senior scientist Poul Jensen from the Department of Conservation, the Danish National Museum.

It is clear that preventing shrinkage is important in the conservation of archaeological wood. Several techniques have been used with different degrees of success through the years. Some methods involve installing a solid substance in the cells to support them when the water is evaporated, two of which are described in the following. Other techniques, such as freeze drying, aim at removing the water from the cells without damaging these in the process, also described below.

1.5.1 The alum method

The alum method was introduced by Herbst[11] in 1861 and it was used in Scandinavia for the 100 years that followed. The waterlogged wood is placed in a warm aqueous solution of alum ($KAl(SO_4)_2 \bullet 12H_2O$) which diffuses into the porous material. When the object is impregnated with alum, it is taken out of the solution and cooled down. Cooling leads to the formation of alum crystals inside the wood cells providing support during drying. The alum method prevents shrinkage well, but the wood is often damaged in the long run as illustrated in Figure 8. This is because alum takes up and gives off crystal water to the air in response to changes in RH. This leads to re-crystallisation of the alum in the object with dimensional change as a result. Since this takes place every time the RH changes, alum treated objects are very sensitive to changes in the indoor climate.

Figure 8. Waterlogged archaeological Hazel wood conserved with alum many years ago. The object is nearly pulverised because it was kept in an environment with fluctuating RH.

1.5.2 Polyethylene glycol impregnation

A conservation method involving impregnation with polyethylene glycol (PEG) was introduced in the 1960's.[12,13] For conservation the water soluble polymer PEG with the general formula $HO(CH_2CH_2O)_nH$, is used in molecular weights between 400 and 4000 g/mol (n = 9 to 91). This polymer melts at room temperature when the molecular weight is near 600 g/mol. The wood is soaked in an aqueous solution of PEG, either by submerging the object into tanks containing the PEG solution or by spraying the object with a PEG solution. Submersion was used in the case of the Skuldelev ships, the Vasa was sprayed. The PEG concentration has to be chosen carefully in order to avoid osmotic collapse of the wood. If the PEG concentration is too high in the beginning of the process, a large difference in osmotic pressure is set up between the water inside the wood and the PEG solution outside. As a result the PEG solution pulls water out of the wood faster than the PEG diffuses into the wood, which leads to collapse. For this reason a low PEG concentration (e.g. 10%) is chosen initially, and then gradually increased to approximately 95% at the end of the impregnation. Passive diffusion alone drives PEG into the wood, thus the process is lengthy. For heavy timbers such as those of the Vasa, 17 years of spraying was required for proper impregnation at room temperature. The rate of diffusion is temperature dependant, if the temperature is increased the impregnation time is shortened. Heat was applied to the impregnation tanks when the Skuldelev ships were conserved. This is not normal practice today because high temperatures are believed to promote degradation of PEG. There has been some variation in the PEG molecular weights chosen for different conservation projects through the years, to an extent these choices were based on intuition. In the conservation of the Vasa, three different PEG types were chosen: PEG 600, PEG 1500 and PEG 4000. It was argued that PEG 600 would bulk the cell wall, PEG 1500 would occupy the lumen and PEG 4000 provide a surface seal.[1] In the conservation of the Skuldelev Viking ships only PEG 4000 was used.[14] When the impregnation is completed, excess PEG is melted off the wood surface using hot air blowers or an oven.

1.5.3 Freeze-drying[15,16]

The surface tension of water inside wood is partly responsible for the collapse that is observed when waterlogged wood is dried. The problem is sometimes circumvented by freeze-drying. Freezing a waterlogged wooden object, eliminates surface tension of the water and thereby capillary forces. Lowering the pressure then allows it to sublimate from ice and the object is dried without shrinkage. Most objects are impregnated a little with low molecular PEG before they are freeze-dried. This is because a moderate PEG-impregnation can improve the strength of the final product somewhat, particularly if it was very fragile to begin with. The freeze-dried objects are lighter and better looking than objects conserved with the conventional PEG-impregnation.

However the use of freeze-drying is limited to objects that can fit into the chamber of the available freeze-dryer. At the Department of Conservation at the Danish National Museum, the largest freeze-drying chamber is 8 m long and 2 m in diameter, the pressure is between 0.05 and 0.1 mbar and the chamber wall temperature between –30 and –35 °C.

2 PEG state and distribution in the Vasa and the Skuldelev ships

When waterlogged archaeological wood is impregnated with PEG, the idea is to get PEG into the wood without causing too much damage to either the PEG or the wood.

There have been documented cases of PEG degradation during conservation of waterlogged archaeological wood. The first reported case was in relation to the re-conservation of the Hjortspring boat.[17] The Hjortspring boat, shown in Figure 9 A, was built of lime-wood approximately 350 BC. The boat was impregnated with PEG 4000 in the 1960's after the old alum impregnation had been removed. Surplus PEG was melted off the many pieces of wood by placing them in an oven at 80 °C for a few hours. It was noticed that the PEG that had come off the wood did not solidify after cooling as it should if it was pure PEG 4000. It was then shown using SEC (Size Exclusion Chromatography) and TLC (Thin Layer Chromatography) that the molecular weight of this PEG had been reduced drastically during the heating in the oven, and that the molecular weight distribution was tailing.

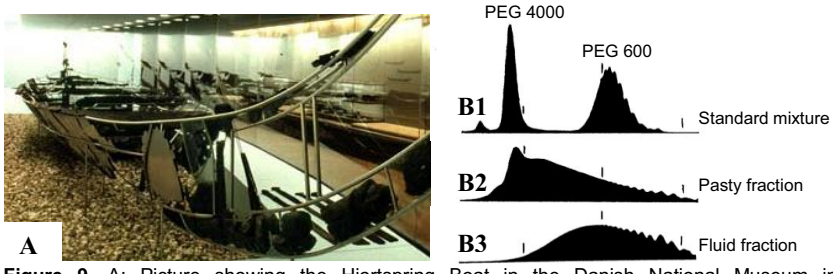


Figure 9. A: Picture showing the Hjortspring Boat in the Danish National Museum in Copenhagen.[18] B: Size exclusion chromatograms of surplus PEG from the conservation of the Hjortspring boat. B1: standard mixture of PEG 4000 and PEG 600, B2: a pasty PEG fraction from the oven. The molecular weight is tailing. B3: fluid fraction with highly decreased molecular weight. The SEC in Figure B is reproduced after Padfield *et al*.[17]

This is illustrated in Figure 9 B. A SEC recording of fresh PEG 4000 mixed with fresh PEG 600 is shown for reference Figure 9 B1. The middle chromatogram (Figure 9 B2) shows a pasty fraction collected after the heating of the impregnated wood. It is clearly seen that the molecular weight of this PEG is tailing downwards from the 4000 g/mol to less than 600 g/mol. A fluid fraction was analysed too (Figure 9 B3), it is seen that this PEG has an even lower molecular weight that is almost centred around 600 g/mol. Since many properties of PEG are closely related to the molecular weight, it became clear that PEG degradation might pose a problem in conservation. This work showed that PEG is less stable than previously assumed and it showed that what was considered a harmless conservation procedure, melting the PEG, resulted in damage to the conservation agent itself.

The re-conservation of the Hjortspring boat showed that heat is a critical factor in relation to the integrity of the PEG-impregnation. The five Skuldelev Viking ships were impregnated with PEG as

described previously. In this project only PEG 4000 was used. The impregnation was done by submerging the planks into large tanks with solutions of PEG 4000. These solutions were heated, at least in some periods of the impregnation, in order to speed up the diffusion of PEG into the wood.[14] Another interesting detail regarding potential hazards to the PEG-impregnation is that the plan for maintaining the exhibited ships, includes procedures involving the use of heat. Once every year the ships were cleaned. Dust was removed using vacuum cleaners. Dirt was washed off from specific parts of the ships using water or alcohol. The cleaned surfaces were then restored by melting fresh PEG 4000 into the wood using hot-air blowers.[19] When comparing these procedures with the procedures described for the Hjortspring boat, it is clear that the Skuldelev ships, too, have received treatments that are hard on the PEG-impregnation.

The treatment of the Vasa also involved procedures that could be considered risky for PEG integrity. Towards the end of the conservation, PEG 4000 was melted into the wood surface using hot-air blowers. It was the idea that this PEG 4000 layer might provide a seal between the wood and the surroundings. If it is assumed that the degradation described for the Hjortspring boat is an oxidation, then there may be another critical step in the conservation of the Vasa. The Vasa was spray treated with aqueous PEG solutions. This way there was plenty of access of air to the PEG in the solutions. On the other hand heat was not involved here so if this is the main cause of degradation, there should not be any problems with degradation in the spray treatment.

The conservation history of the Vasa is described in Håfors.[1] It explains the choices of PEG types and the order of application. Three different PEG types were used in the impregnation, namely PEG 600, PEG 1500 and PEG 4000. The PEG treatments were initiated in 1962 with application of aqueous PEG solutions to various parts of the ship. This was done manually from 1962 to 1965 and both PEG 1500 and PEG 4000 were applied in this period. In 1965, the automatic spraying system was put into use. This covered the entire ship in a mist of aqueous PEG solution. Initially PEG 1500 was used in these solutions, but in 1971 it was decided to change to PEG 600. The PEG 1500 had not gone as deep into the wood as expected. PEG 600 was used instead of PEG 1500 when new PEG was added to the spray solutions. It was believed that PEG 600 would have the same favourable properties as PEG 1500 regarding dimensional stabilisation of the wood but the smaller molecule was expected to migrate into the wood faster than the larger PEG 1500. PEG 600 was used in the automatic spray system until it was turned off in 1979. PEG was still applied to the ship locally from 1979 to 1989, but this was done manually. Application of both PEG 600 and PEG 4000 has been documented in this period. Thus investigating the PEG-impregnation of the Vasa today sheds light on both the stability of PEG and the diffusion of the individual PEG types into the wood material.

The PEG-impregnation in the Vasa and the Skuldelev ships was investigated now almost half a century after the impregnation began. It was the idea to try to use modern analytical techniques to investigate the current state of the PEG. PEG extracted from the ships was analysed to see if the molecular weight distribution looked as degraded as it did for the PEG from the Hjortspring boat conservation project. It was also the idea to try to look at the distribution below the wood surface of the different PEG types used on the Vasa to see if this matches the order of application of the PEG types during PEG treatment. The results of these investigations are described in an article published in the Journal of Archaeological Science in 2007. Furthermore, this chapter give results that supplement the article. The article, referred to as Article I, is printed in Appendix 1.

2.1 Further experimental

ATR FT-IR (Attenuated Total Reflectance Fourier Transform-InfraRed) spectra were collected over 30 scans at a resolution of 4 cm^{-1} between 4000 cm^{-1} and 600 cm^{-1} (the lower limit of sensitivity for ATR) using an ASI DurasamplIR 1 single reflection accessory with an angle of incidence of 45° and fitted with a diamond internal reflection element in a Perkin–Elmer Spectrum 1000 FTIR spectrometer. The refractive properties of the diamond in the reflective cell allow absorbance data to be collected from a depth approximately equal to that of the wavelength of the infrared radiation, a maximum depth of approximately 2 μm. Background spectra of the empty, clean accessory open to air were run at hourly intervals. The quality of spectra was highly dependent on achieving close contact between the DurasamplIR 1 reflection element (the diamond crystal had an active area of 1 mm diameter) and the surface of the wood discs described in Article I. Identical pressure distribution was achieved for all samples using a torque limiter.

ESI MS (Electro Spray Ionisation Mass Spectrometry) was performed on a Q-TOF tandem mass spectrometer (Q-TOF Ultima Global, Micromass UK). A highly diluted solution of the sample PEG extract, was prepared in methanol/water 1:1 containing 20 mM ammonium formate. The sample solution was sprayed into the instrument through an Atmospheric Pressure Inlet (API). In contrast to MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry), ESI often gives rise to multiply-charged ions. PEG sometimes carries as much as four charges. This makes the spectra very crowded. However the regions of PEG 600 and the high mass end of PEG 1500 are relatively free of multiply-charged species in the sample matrices relevant here. Artefacts relating to ammonium formate such as formates on PEG have not been observed when fresh PEG samples were run.

Sampling and sample treatment is described in Article I. The extraction procedure, the procedures regarding MALDI-TOF MS and SEC (Size Exclusion Chromatography) are also described in Article I. A mass calibration curve of PEG was made with the SEC columns that were used, this is given in Appendix 4.

2.2 Further results and discussion

Infrared spectra were recorded on the surface of the sample wood discs described in Article I, using an ATR FT-IR instrument. These results are summarised in Figure 10 where the upper two spectra are of fresh European oak wood first and pure PEG 4000 second.

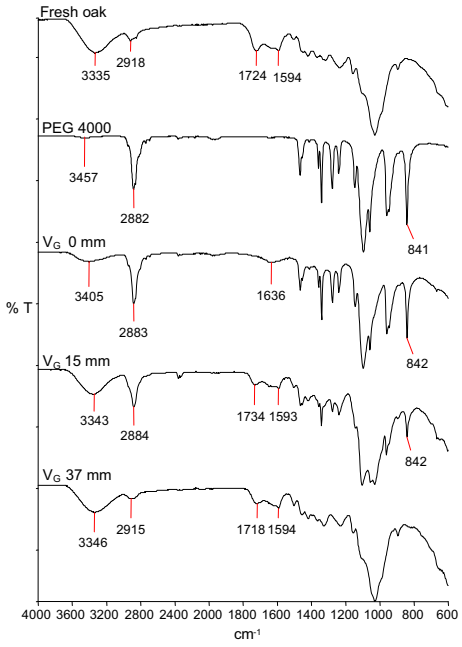


Figure 10. ATR FT-IR recordings (from top): fresh European oak, PEG 4000, V_G at the surface, V_G 15 mm below the surface and V_G 37 mm below the surface of the sound Vasa sample (V_G). The signature of PEG is dominant at the surface of V_G (0 mm) whereas the signature of oak is dominant in the core of V_G (37 mm).

Oak and PEG have some overlapping bands between 1500 cm^{-1} and 900 cm^{-1}, but outside this region there are several characteristic bands. Cellulose and lignin in oak have OH stretch at 3335 cm^{-1}, PEG has a weak OH band, too. CH stretch is seen around 2900 cm^{-1} for both oak and PEG, but more intensely for PEG. Oak has two bands at 1724 cm^{-1} and 1594 cm^{-1} which have been assigned to carbonyl C=O stretch in hemicellulose and ring deformation in lignin, respectively.[20] Around 841 cm^{-1} symmetrical C-O-C stretch is seen in the spectrum of PEG. This band is unique for PEG. Spectra recorded 0, 15 and 37 mm under the wood surface of a Vasa sample that has been described as being in good condition (V_G) are shown in Figure 10. The spectrum recorded at the surface (V_G 0 mm) is almost identical to the PEG spectrum. It has a weak OH band, the CH band is intense, no carbonyl or aromatic ring deformation is seen and the $PEG_{C\text{-}O\text{-}C}$ band is intense. This is in agreement with the surface wood being full of PEG as demonstrated by extraction experiments in Article I. The spectrum recorded at 37 mm is dominated by the signature of the oak spectrum with its intense OH band, weak CH band, signals for the carbonyl, the aromatic ring deformation and no $PEG_{C\text{-}O\text{-}C}$ signal. Thus it seems that there is very little PEG at this depth, which is also in good agreement with the extractions described in Article I. The spectrum recorded 15 mm below the surface looks like a combination of the oak and the PEG spectra, it has all the signals mentioned. OH, CH, C=O, aromatic ring deformation and C-O-C all appear with intermediate intensity indicating that the sample contains some PEG and some wood. Thus the PEG concentration decreases from the surface to the centre of the wood. This trend is observed for the samples that were in good condition (V_G and S). However for the samples that

were visually deteriorated (V_M and V_D) the PEG-bands were intense at all depths meaning that these samples have a high concentration of PEG throughout. This makes sense for wood that was degraded before impregnation. Degraded wood is more porous than intact wood, therefore more PEG enters the degraded regions. V_D and V_M were degraded at all depths and ATR FT-IR shows that they contain PEG all the way through (data not shown). V_G and the Skuldelev sample were more degraded at the surface layers and, for these samples, this is where PEG was detected. ATR FT-IR was useful in tracing PEG and wood in samples from the Vasa and the Skuldelev ships. It could also have a potential for monitoring PEG content in wood during impregnation.

Many MALDI-TOF mass spectra recorded on PEG extracts from the wood samples and the pre-treatment of these samples (including mixing with the sodium chloride and dihydroxy benzoic acid matrix) are presented in Article I. The spectra show that the PEG molecular weight distribution is tight and symmetric in most samples but that a few samples have “tailing” molecular weight distributions. These tails could represent degraded PEG, assuming that the molecular distribution was narrow and symmetric in the PEG that was used for impregnating the ships in the first place. However, if the PEG used on the ships had a tailing molecular weight distribution to begin with, then this molecular weight profile should reflect in the wood somehow. This would not be an accurate image of the molecular weight profile of the original PEG because the small molecules should diffuse faster into the wood than the larger ones. Thus small molecules should be more readily represented than larger molecules at large depths under the wood surface and larger molecules readily represented near the surface.

Samples of PEG leftover from the conservation of the Vasa were recently discovered in a storage facility on Beckholmen in Stockholm. A few of these samples were analysed using MALDI-TOF MS. Figure 11 shows two of these recordings.

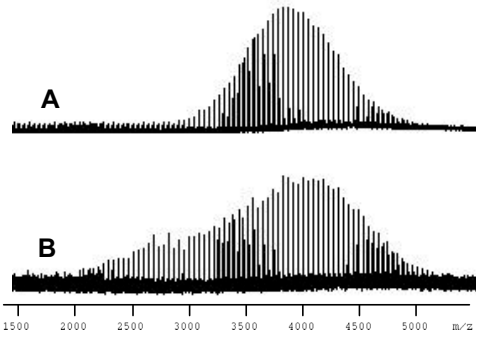


Figure 11. MALDI-TOF mass spectra recorded on PEG leftovers from the conservation of the Vasa, found on Beckholmen Stockholm. A: PEG 4000 supplied by Berol AB. B: PEG 4000 supplied by Mo & Domsjö AB.

Figure 11 A shows a MALDI-TOF MS recording of PEG 4000 supplied by the company Berol AB. It is seen that the molecular weight distribution is fairly tight and symmetric. This is how one would imagine the molecular weight distribution in a batch of new PEG. In Figure 11 B a MALDI-TOF MS recording of PEG 4000 supplied by Mo & Domsjö AB is shown. Here the molecular weight distribution is broader and the distribution is tailing downwards to the low *m*/*z* values. This could be

due to degradation before delivery from the supplier or maybe to the mixing of two batches of PEG 4000 with slightly different average molecular weights. No matter what the explanation might be, some of the PEG that was used on the Vasa has a tailing molecular weight distribution. This could be a source of confusion when looking at MALDI-TOF mass spectra such as in Article I. Now tailing molecular weights in PEG from the Vasa is not enough evidence in itself, to say that PEG degradation has taken place because the tailing could have been in the PEG from the beginning. One last option is that the PEG in Figure 11 B, has simply degraded during the time it has been sitting in Beckholmen. However, this seems unlikely because the other sample from Beckholmen (Figure 11 A) which does not have tailing, has been standing in the same room for as long as the tailing PEG sample. It is also seen in the Vasa that most PEG samples have perfectly tight molecular weight distributions. PEG in the Vasa might also have degraded if PEG in a storage room has degraded over the same time period. In summary the finding that some of the PEG used for impregnation of the Vasa shows tailing of the PEG molecular weight distribution makes it harder to trust conclusions regarding PEG degradation in the Vasa that is based on evaluation of molecular weights and molecular weight distributions alone.

A number of MALDI-TOF MS recordings have been done on PEG extracted from PEG-treated archaeological wood by this group and by others.[21] In a few cases “satellite peaks” or peaks in between the parent PEG signals, were observed. It has been very tempting to interpret these as PEG degradation products or at least as modified PEG. It was attempted to reveal the identity of these ions by CID (Collision Induced Dissociation) experiments on an ESI mass spectrometer. However this was not possible due to lack of intensity in the satellite peaks. Another possibility is that the signals are generated in the MALDI-TOF MS or in connection with the sample preparation. In Figure 12 parts of two MALDI-TOF mass spectra are shown. They are both recorded on a PEG extract from the Vasa (V_M). In the first, 45 % of the available laser power in the MALDI apparatus was applied. In the second, 30 % of the laser power was applied.

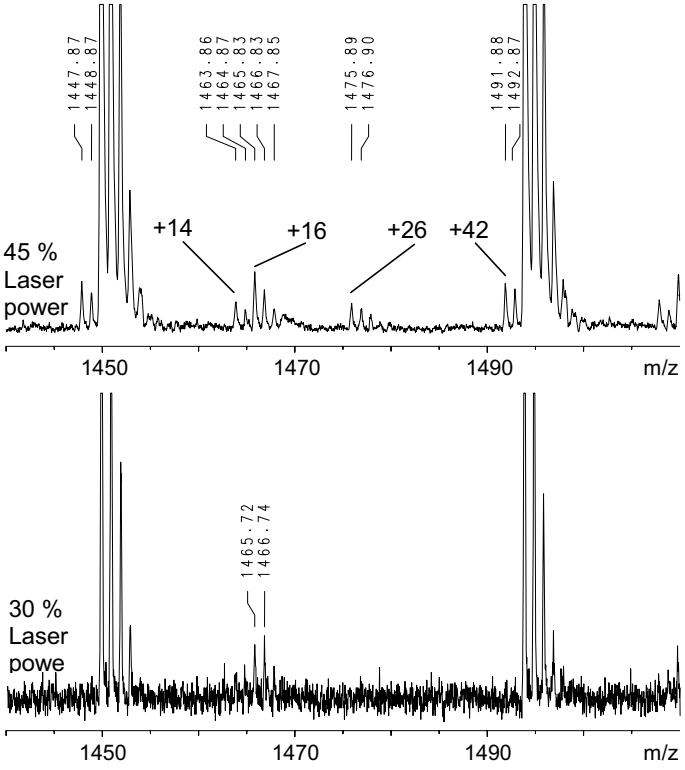


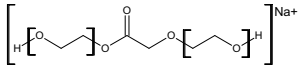
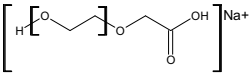
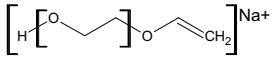
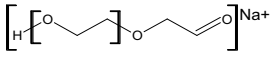
Figure 12. MALDI-TOF MS recordings on PEG extracts from the slightly degraded Vasa sample (V_M). Singly charged satellite peaks are observed at 14, 16, 26 and 42 mass units over the parent PEG ion assigned to $[PEG]Na^+$. Top: spectrum recorded at 45% laser power. Bottom: spectrum recorded at 30 % laser power, sample and range are identical.

Several satellite peaks are observed in the recording at 45 % LP. They are found at +14, +16, +26 (or -18) and +42 (or -2) mass units relative to the parent PEG ion ($[PEG]Na^+$). The satellite at *m*/*z* +14 and part of the one at *m*/*z* +16 can be explained by the sodium chloride cluster of PEG, $[PEG\ NaCl]Na^+$. Due to the characteristic isotopes of halogens, this ion has a signal at *m*/*z* +58 and *m*/*z* +60 over the parent ion $[PEG]Na^+$, which corresponds to *m*/*z* +14 and *m*/*z* +16 over the PEG ion one repeating unit larger than the parent ion (the spacing is *m*/*z* 44). *m*/*z* +16 can also be achieved by substituting the sodium ion with a potassium ion. Potassium ions are frequently seen as contaminants in reagents used in the MS lab, e.g. in sodium chloride which is added to the sample to provide a suitable cation for the sample molecules. The added sodium chloride also makes sodium chloride salt cluster formation favourable. Thus there is an obvious explanation for the peaks at *m*/*z* +14 and *m*/*z* +16. Clearly there has been some discussion whether the signals could be due to a modification of the PEG. The *m*/*z* +14 can be satisfied by adding CH_2 to the formula or by adding O and subtracting H_2. Rings such as, e.g., in-chain dioxane, propylene units and methyl ethers on PEG are a few options if satisfying the *m*/*z* is the only concern. However, carbonyl groups on the PEG chains are considered more realistic from a chemical point of view. These would be carboxylic acids or in-chain esters as shown in Table 1. A formula with *m*/*z* 16 over the

parent [PEG]Na^+ ion can be achieved by adding an oxygen atom to the formula. Theoretically this could be as an extra ether oxygen atom in the PEG chain, it could also be as an additional alcohol. The alcohol would either be as a hemiacetal (in-chain) or as a geminal diol (at the end group of the PEG). Hemiacetals are unstable and the geminal diols are more likely to appear as the corresponding aldehyde. Thus m/z +16 is most likely due to the salt clusters or potassium ions already discussed (Table 1).

The peak at *m*/*z* +26 (or –18) can be explained by dehydration of PEG in the mass spectrometer. This could lead to ring formation or more likely vinyl ethers as shown in Table 1. The satellite at *m*/*z* +42 (or -2) is explained by loss of hydrogen from PEG in the mass spectrometer. This results in unsaturations in PEG such as double bonds, rings or aldehydes. It is also possible to explain the *m*/*z* +42 satellites with a potassium ion on the +26 species, since the mass difference between *m*/*z* +26 and *m*/*z* +42 is 16. However it seems that the most probable assignment is the aldehyde. Both of the reactions behind the *m*/*z* +26 and *m*/*z* +42 represent loss of neutrals and they are common reactions in the mass spectrometer. This is illustrated further in the lower part of Figure 12 where 30 % LP was used in the ionization of the same sample. Here almost all of the satellite peaks have disappeared, only a little peak is left at *m*/*z* +16. It is also seen that the signal has become weaker. Thus it may be that the more intense ionization at 45 % LP is what creates the ions behind the satellite peaks, but it may also be that the intense ionization is necessary to make the named ions desorb if they are in fact part of the sample.

Table 1. Overview of possible assignments for satellite peaks in MALDI-TOF MS of PEG from the Vasa and Skuldelev 2.

***m*/*z* +14**	***m*/*z* +16**	***m*/*z* +26 (-18)**	***m*/*z* +42 (-2)**
Salt cluster [PEG NaCl]Na^+ In-chain esters  Carboxylic acids 	**Salt cluster** [PEG NaCl]Na^+ **Potassium ion** [PEG]K^+	**Vinyl ether** 	**Aldehyde** 

Species with boldface headlines are considered realistic.

The salt clusters at *m*/*z* +14 and *m*/*z* +16, PEG with a potassium ion at *m*/*z* +16, the dehydrations for the *m*/*z* +26 satellite and loss of hydrogen in the *m*/*z* +42 peak are the assignments considered most realistic here. They are marked with a boldface headline in Table 1. The most probable explanation for the satellite peaks is formation in the MALDI mass spectrometer or in reactions with the MALDI matrix.

Thus it seems that the use of MALDI-TOF MS as a technique to detect products of PEG degradation as satellite peaks could be risky. If e.g. in-chain esters were part of PEG in a sample of PEG extracted from the Vasa or the Skuldelev ships, then it would be hard to know that the *m*/*z*

+14 was due to this in-chain ester and not just a sodium chloride salt cluster. At least supplementary investigations such as CID would have to follow.

2.3 Further conclusions

ATR FT-IR measurements confirmed that the content of PEG in a sound sample from the Vasa had high amounts of PEG in the surface layers and small amounts of PEG deeper inside the wood. The technique could be useful for measuring the PEG content in waterlogged archaeological wood in the conservation process.

Samples from some of the batches of PEG that had been used for conserving the Vasa were analysed with MALDI-TOF MS. Some of them contained PEG with tailing molecular weight distributions while other samples had more regular molecular weight profiles. The finding that some of the PEG that had been used to impregnate the Vasa, contains PEG with tailing molecular weight distributions, makes it harder to use tailing molecular weights as an indication for PEG degradation in the Vasa timbers and maybe in general. Degradation does cause tailing PEG molecular weight distributions, but tailing molecular weight distribution alone does not prove that degradation has taken place.

Sodium chloride salt clusters and potassium ions on PEG are proposed as the most likely explanation for the satellite peaks at m/z +14 and m/z +16 observed in MALDI-TOF MS recordings of PEG extracts from the Vasa and the Skuldelev ships. Satellite peaks at m/z +26 and m/z +42 were observed too and it was shown that they are probably results of too intense ionization in the MALDI. Using MALDI-TOF to detect PEG reaction products as satellite peaks may be risky.

3 Accelerated ageing of PEG and TEG - the effect of matrix

Polyethylene glycol (PEG), also known as polyethylene oxide (PEO) when the molecular weight is high, is manufactured in molecular weights ranging from 200 to 35000 g/mol.[22] It is produced by anionic ring opening polymerisation of oxirane (ethylene oxide) as illustrated in Figure 13, initiated by water.[23]

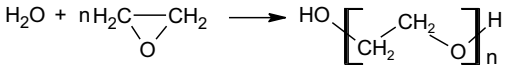


Figure 13. Synthesis of PEG by anionic oxirane ring opening polymerisation initiated by water. This reaction is used in the manufacture of PEG by Clariant. n is the number of repeating units.[23]

PEG is used to impregnate waterlogged archaeological wood, as explained previously. However, the main applications of PEG, from an industrial point of view, are: as tablet binder, as an additive in cosmetics, paint, paper, and high strength concrete and as a lubricant in rubber moulding.[24] Clearly the stability of PEG is important in relation to its performance in all the individual applications. For this reason, PEG stability has been studied from many different perspectives.

3.1 Polyethylene glycol degradation

3.1.1 Thermo-oxidative degradation

The fact that PEG is degraded in the presence of air at elevated temperatures is well established. Heat and air have been applied to PEG and the change in PEG molecular weight over time has been measured more or less directly by techniques such as differential scanning calorimetry (DSC) (change in T_G), viscosimetry, size exclusion chromatography (SEC), solution density and refractive index.[25-28] The studies show that PEG degrades in the presence of air and heat. This is illustrated using viscosimetry in Figure 14. The viscosity, and thereby the molecular weight, of PEG decreases as a function of time of exposure to air and heat.

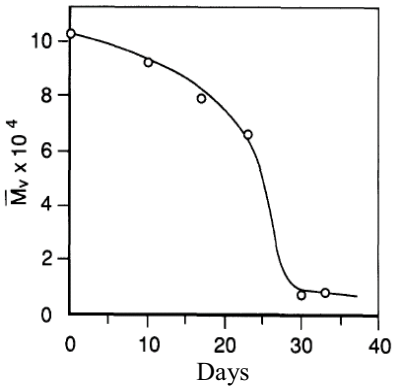


Figure 14. Viscosity-average molecular weight ($\overline{M}_V$) of PEG as a function of ageing time (days) at 60°C in the presence of oxygen. After Scheirs *et al.*[29]

Other techniques have been applied that yield a little more information about the chemistry of the degradation process, they include pH measurement, oxygen consumption, saponification number and thermo-gravimetry (TG).[29-31] More indirect evidence for PEG degradation has also been presented. Drugs in tablets with PEG as the binder sometimes degrade as a consequence of PEG instability; PEG-hydroperoxides appear to play an important role.[32,33] In summary these investigations show that PEG is degraded in the presence of air and heat. Furthermore they show that acidic degradation products are formed (possibly carboxylic acids from esters) and they show that oxygen is consumed in the process. An example of a study of PEG degradation during conservation was given in the introduction in Chapter 2. This concerned the re-conservation of the Hjortspring boat in the 1960's.[17]

The first real attempts at elucidating the mechanism of thermo-oxidative degradation were published in the mid-1960's.[34-36] Goglev *et al.*[36] found that water and formaldehyde were the most prominent degradation products at 92 °C and PO_2=400 mmHg (53 kPa) and that PEG-hydroperoxides were intermediates in the process. The compounds were identified by mass spectrometry and quantified by chromatography. The hydroperoxides were quantified by titration. The change in concentration with time of degradation is shown in Figure 15.

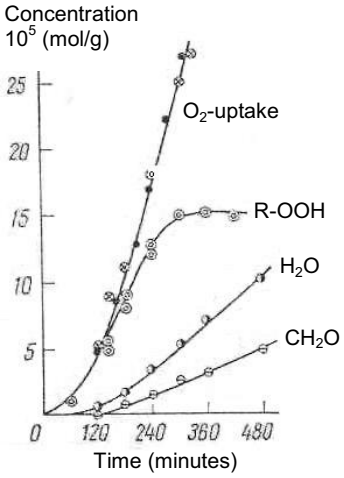


Figure 15. Oxygen uptake and concentration of degradation products (hydroperoxides, water and formaldehyde) during the degradation of PEG at 92 °C and PO_2=400 mmHg (53 kPa), after Goglev *et al.*[36]

In the beginning of the reaction, all the consumed oxygen goes into formation of hydroperoxides; the other products only start to form when the hydroperoxide concentration begins to deviate from the oxygen uptake. This shows that the hydroperoxide is the primary product in the reaction and that it is degraded itself in reactions that lead to the formation of formaldehyde and water. Other volatile products were detected in smaller quantities during PEG degradation at temperatures between 92 and 162 °C. Besides water and formaldehyde, small amounts of CO_2, CH_3CHO, $HCOOCH_3$ and $HCOOC_2H_5$ were detected. To account for these products a mechanism was proposed by Goglev *et al.*[36] (Figure 16) in which a dihydroperoxide is formed through a six membered intermediate.

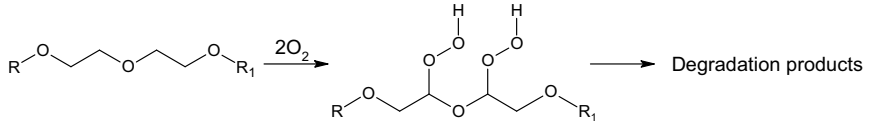


Figure 16. A dihydroperoxide of PEG is proposed as the primary intermediate in the degradation of PEG in the mechanism proposed by Goglev *et al.*[36] R and R_1 represent the remaining part of the polymer (-[CH_2CH_2O-]$_n$-H).

The dihydroperoxide breaks down to form the identified products. However, the stoichiometry between two products (H_2O and CO_2) could not be accounted for. The non-volatile (polymeric) products were shown to contain a carbonyl group using IR spectroscopy; the carbonyl oxygen originates from the ^{18}O enriched gas phase because the C=^{16}O stretch peak shifted down by 30 cm^{-1} for the products formed in ^{18}O enriched gas corresponding to the C=^{18}O stretch frequency.

Heatley and co-workers[37-39] have studied the non-volatile residue left after thermo-oxidative degradation of PEG (PEG750 monomethoxy, PEG $4*10^6$, PEG 2000 monomethoxy, PEG 5000 monomethoxy and PEG 20000) at 150 °C for about 4 days. The residue was studied using ^1H and ^{13}C NMR and a range of 2D-NMR techniques. The functional groups identified are listed in Table 2 in order of abundance.

Table 2. Functional groups identified in the solid residue after thermo-oxidation (150 °C) for 4 days of mono methoxy PEG 5000. The compounds are listed with the most abundant species first. R represents the remaining part of the polymer.

Formate	$-CH_2OCH=O$
In-chain ester	$-OCOCH_2O-$
Hydroxyethyl methanoate	$HOCH_2CH_2OCH=O$
Ethylene glycol dimethanoate	$O=CHOCH_2CH_2OCH=O$
Ethanoate ends	$-CH_2OCOCH_3$
Acetal link	$-O-CH_2-O-$
Peroxy species	RCH_2OOH

The functional groups support a mechanism (Figure 17) where a hydroperoxide rearranges to give a formate end group and a hemiacetal; the latter is further split up into formaldehyde and an alcohol.

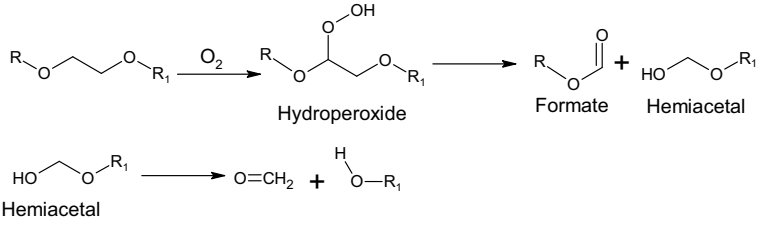


Figure 17. Mechanism proposed by Heatley and co-workers.[37-39] A hydroperoxide of PEG reacts to give a formate and a hemiacetal. The hemiacetal is itself unstable and breaks up to yield formaldehyde and an alcohol end group on the polymer. R and R_1 represent the remaining part of the polymer ($-[CH_2CH_2O-]_n-H$).

In an internal report[40] from a manufacturer of PEG (Höechst), triethylene glycol degradation was demonstrated at 120 °C and 1 bar O_2, and 25 °C at 8 bar O_2 over 25 days. Techniques such as GC (Gas Chromatograhy), NMR (Nuclear Magnetic Resonance), ionchromatography, saponification number, OH-number and I_2-number indicated that mono-, di- and triethylene glycol, along with the mono- and diformates thereof were formed. Furthermore water and formic acid were identified as products of the degradation.

Triethylene glycol and tetraethylene glycol were used as model molecules for polyethylene glycol by Glastrup.[41,42] GC-MS (Gas Chromatography-Mass Spectrometry) analysis was used to monitor the degradation at 70 °C for 10 days under constant bubbling (10 ml/min) with either dry or moist (RH=75%) air or nitrogen. For ageing of tetraethylene glycol in dry air it was observed that tri-, di- and monoethylene glycol was formed, as well as some of the mono- and diformates of these

glycols (although not all). Formic acid was also identified. Under nitrogen, no degradation took place. In moist air, the process was slower than under dry conditions. The reaction products were also different; no formates were detected under moist air. This is attributed to ester hydrolysis in the aqueous and acidic environment of the reaction mixture. The mechanism proposed to account for this is essentially the same as that in Figure 17. Three modified triethylene glycols were degraded under the same conditions as the tetraethyleneglycol. The four compounds, illustrated in Figure 18, were not equally resistant to thermo-oxidation at 70 °C.

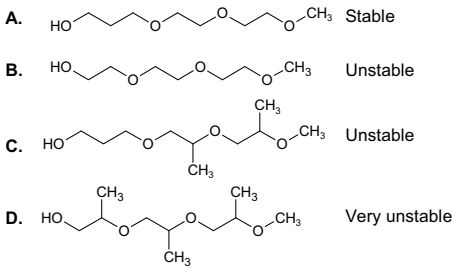


Figure 18. Different modifications of triethylene glycol and their relative stability under degradation at 70 °C with a flow of 10 ml dry air pr. minute.[42]

The propylated version of triEG (A) is the most stable molecule of them all, which is taken as an indication that degradation takes place at the hydroxyl ends of the molecules since this is the only difference between A and B. The molecules C and D are substituted analogues to A and B. B and C degraded almost at the same rate, D degraded faster than the three other species.

The different thermo-oxidative mechanisms proposed and the various degradation products identified are not identical in all the experiments reported. However, the experimental conditions were not identical either. Temperature, oxygen pressure, PEG molecular weight, ageing of aqueous PEG solutions versus solid films or melts are just a few important factors that were different in the experiments presented above. Just as important to the result is the experimental setup. In some cases, ageing was done in an autoclave which allows analysis of volatile products. Other experiments were performed on a residue left in an open vial after 24 hours of heating at 150 °C. Clearly the latter would miss a degradation product like water since it would evaporate under the given conditions. In spite of this, there seems to be a recurring motif: some sort of hydroperoxide seems to fit into the mechanisms as a first intermediate. Another common observation is the formation of carbonyl compounds such as formaldehyde, formic acid, formates, carbon dioxide or in-chain esters.

3.1.2 Radiation induced degradation

A range of investigations have been done on inducing PEG degradation with different kinds of radiation such as γ-radiation,[43-53] light ($\lambda \leq 300$ nm,[54-56] $\lambda = 266$ nm[57]) and microwaves.[58] Clearly microwaves and γ-radiation are not relevant to a museum environment, but it is interesting to note that the products formed from degradation of triethylene glycol in air, initiated by exposure to γ-radiation are, to a large extent, the same as for thermo-oxidation. Marchal and co-workers have identified formic acid and formaldehyde, mono-, di-, and triethylene glycol along with formates and

aldehydes of mono-, di-, and triethylene glycol, as products of triethylene glycol degradation at 25 °C in aqueous solution exposed to γ-rays.[43-50] It seems that much the same kind of bond homolysis results from both heat and irradiation.

Morlat and Gardette have studied the influence of light and heat (50 °C) on the oxidative degradation of PEG (100000 and 4000000) as solid films and in aqueous solution. IR spectroscopy and photo-DSC (photo - Differential Scanning Calorimetry) was used to monitor the development of peaks, mostly in the carbonyl region, during degradation.[54-56] The formation of ammonium formate and formamide, determined by IR, when the aged samples were treated with ammonia, clearly demonstrates that there is formic acid in the sample. A mechanism was proposed in agreement with the production of formates, in-chain esters, carboxylic acids and alcohols. C-H bond homolysis in PEG allows oxygen to be inserted between the carbon and the hydrogen atoms in PEG thereby forming a hydroperoxide. This hydroperoxide is split by O-O bond homolysis to form a hydroxyl ($HO^{\bullet}$) and an alkoxyl radical on the PEG ($-OCH_2CHO^{\bullet}$ O-) which undergo β-cleavage yielding a formate and an alkyl radical ($-OCH^{\bullet}_2$). The alkyl radical is proposed to react with oxygen in a reaction that ultimately leads to a formate.

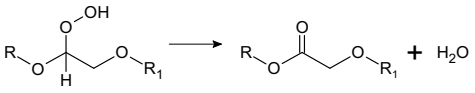
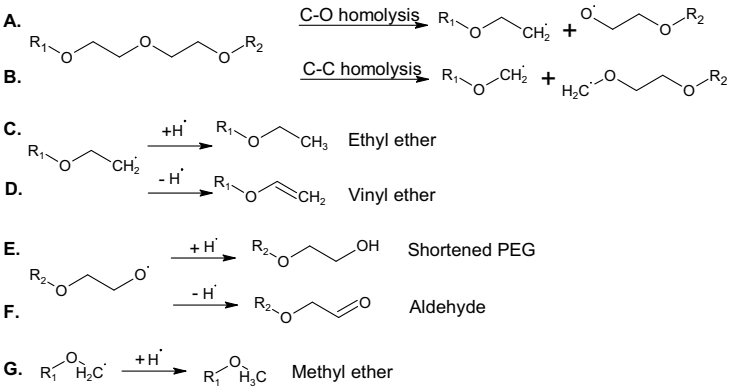


Figure 19. Formation of in-chain ester by dehydration of hydroperoxide.[54] R and R_1 represent the remaining part of the polymer ($-[CH_2CH_2O-]_n-H$).

In-chain esters form by dehydration of the hydroperoxide as shown in Figure 19. This is in good agreement with results from the ^{18}O experiments conducted by Goglev *et al.*[36] which indicated that the carbonyl oxygen atom primarily originates from dioxygen in the atmosphere.

3.1.3 Pyrolytic degradation

Degradation of PEG by heat in the absence of oxygen, pyrolysis, has been investigated thoroughly.[59-66] In one of the latest studies,[64] it was shown that temperatures as low as 150 °C led to pyrolysis of PEG 2000 in an argon atmosphere. The degradation products were detected by mass spectrometry. The reactions proposed that yield these products are outlined in Scheme 1. Degradation starts with homolysis of a C-O (A) or a C-C (B) bond in the PEG. The radicals thus formed undergo intermolecular loss or gain of hydrogen atoms to give ethyl ethers (C), vinyl ethers (D), shortened PEG (E), aldehydes (F) and methyl ethers (G).



Scheme 1. C-O and C-C bond homolysis (A-B) and subsequent hydrogen atom abstractions and donations (C-G) leads to the observed PEG pyrolysis products. Modified after Lattimer.[64] R and R_1 represent the remaining part of the polymer ($-[CH_2CH_2O-]_n-H$).

Since pyrolysis takes place at temperatures as low as 150 °C, it could be contributing to the degradation in the thermo-oxidative reactions too, at least the ones at high temperatures.

3.1.4 Stabilisation of PEG

Since PEG finds so many different uses, the idea of adding some antioxidant to protect it from thermal and oxidative stress is not new.[25,61,67-70] The compounds applied as antioxidants here resemble the commercially available hindered phenols, and many of them seem to work well for PEG stabilisation. Hindered phenol antioxidants work by reacting stoichiometrically with different radical species to form stable products. For example, peroxy radicals ($ROO^{\bullet}$) are removed by hindered phenols via hydrogen atom ($H^{\bullet}$) donation as illustrated in Figure 20 for butylated hydroxytoluene (BHT).

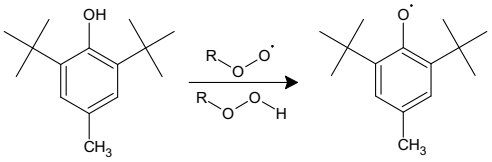


Figure 20. Hindered phenols (here BHT) remove peroxy radicals through hydrogen atom donation.[71] R represents the remaining part of the polymer.

The phenoxy radicals generated are resonance stabilised. As illustrated in Figure 21, the phenoxyl radicals can react with other radical species such as alkoxyl radicals ($RO^{\bullet}$). This can result in the attachment of an alkoxyl radical to the antioxidant phenoxyl radical as illustrated in Figure 21A, or a second hydrogen atom can be abstracted from the phenoxyl radical by an alkoxyl radical generating the quinone methide illustrated in Figure 21B.

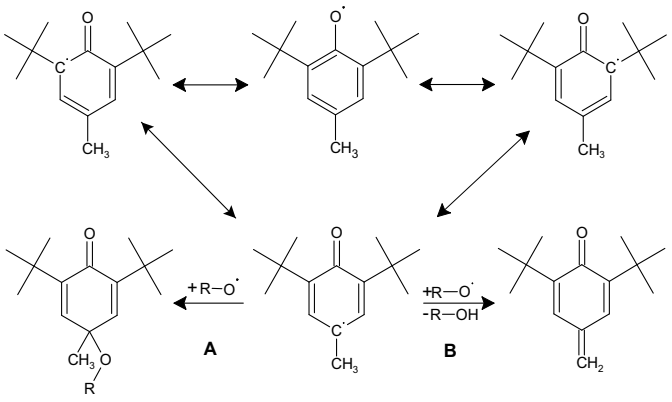


Figure 21. Phenoxy radicals, in this example of buthylated hydroxy toluene (BHT), react with alkoxyl radicals (RO•) to produce either an adduct (A) or to donate a hydrogen atom to the alkoxyl radical (B). R represents the remaining part of the polymer.[71]

When radicals are removed quickly like this they do not have much time to damage the polymer, which is then protected. There are many different commercially available antioxidants, hindered phenols are just one example.

It has been known for many years that certain salts affect the rate of degradation of PEG.[25,72-74] For example, Costa *et al.*[72] described a thermogravimetric experiment at 322°C under air. Here potassium iodide, and several other iodides, slowed down the degradation of PEG. Another example is the experiment reported by Lloyd *et al.*[74] in which the effect of 8 different salts on diethylene glycol was tested. It was demonstrated that, e.g., iron(III)chloride provides good protection in an experiment conducted at 75 °C with an oxygen pressure of 1 atmosphere. The experimental conditions may be a little harsh compared to the conditions in a museum but the fact that certain salts affect PEG stability and that others do not, seems important. The mechanisms suggested by the two authors are far from identical but both involve hydroperoxides and free radicals in the polymer.

The present work also deals with stabilization of PEG. Degradation of TEG (TetraEthylene Glycol) and PEG was investigated under air and under nitrogen at 70 °C. The processes were investigated on their own and in the presence of small amounts of different additives. These additives were: salts known to have an effect on the degradation, salts that had not been investigated before in this respect and salts that were relevant to archaeological wood because they had been identified in archaeological wood previously. The degradation was monitored by the weight of the TEG and by various analytical techniques such as GC-MS and ATR FT-IR (Attenuated Total Reflectance Fourier Transform-InfraRed).

The results from these experiments are given in a manuscript for an article that will be submitted to Journal of Archaeological Science. This text is referred to as Article II, and it is printed in Appendix 2. Results that are not in the manuscript are detailed in the following pages.

3.2 Further experimental

The instruments used for the experiments in this section have been described elsewhere. MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry) was described in Article I, ESI-MS in section 2.1, ATR FT-IR in Article II and GC-MS in Article II. The setup used for accelerated ageing of TEG was described in Article II as well. In spite of this, an illustration of the setup for accelerated ageing of TEG is given in Figure 22. It shows a drawing of the reaction vial (A) containing TEG at 70 °C which is connected to a condenser vial (B) kept at – 10 °C.

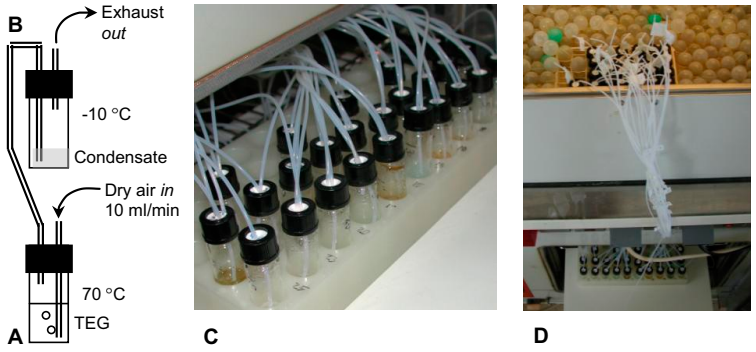


Figure 22. A and B: drawing of the experimental set-up for accelerated ageing of TEG. The reaction vial (A) contains TEG and 10 mM of an additive. The temperature is 70 ºC, dry air bubbles through the liquid. The condenser (B) is placed in a cryostatic bath at max - 10 ºC so that volatiles from the reaction in A condense here. C: picture of the 25 reaction vials used in the experiment. D: picture that show the 25 reaction vials in the oven (bottom) connected to the 25 condensers in the cryostatic bath (top).

Dry air or nitrogen was bubbled through the liquid in the reaction vial at a rate of 10 cm^3/min. Volatile reaction products leave the reaction vial through a Teflon tube and condense in the condenser vial. Picture C shows the 25 reaction vials that were available in this experiment. They were placed in an oven and connected to the condensers, one and one, in the cryostatic bath as shown in D.

3.3 Further results and discussion

3.3.1 Accelerated ageing of TEG

It was mentioned in Article II that reaction vials and condenser vials were analysed with GC-MS and ATR FT-IR to get information about the change in TEG (TetraEthylene Glycol) concentration over time and about the identity of reaction products. Figure 23 shows gas chromatograms recorded after 0, 11 and 14 days of ageing of pure TEG at 70 °C under air. In the chromatogram recorded after 0 days of ageing, an intense peak corresponding to TEG is seen at retention time 10.04 min. The internal standard used was naphthalene and it is seen at 6.04 min.

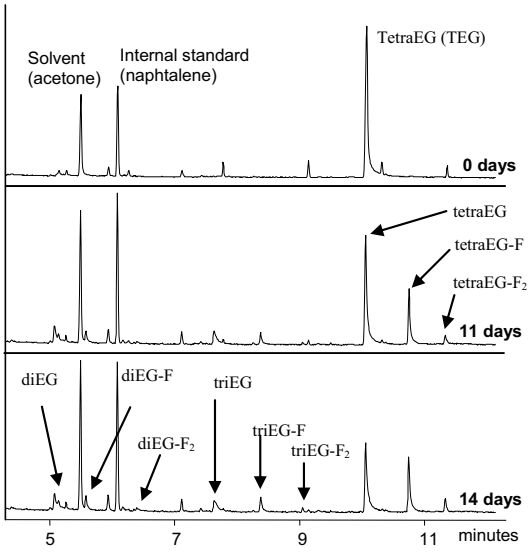


Figure 23. GC-MS recordings of samples collected from the reaction vial containing pure TEG in air after 0, 11 and 14 days. As tetraethylene glycol (*tetra*EG) is consumed, mono and di- formates thereof are formed (tetraEG-F and tetraEG-F_2). Tri- and diethylene glycol (*tri*EG and *di*EG) as well as the mono- (*tri*EG-F and *di*EG-F) and diformates thereof (*tri*EG-F_2 and *di*EG-F_2) are also detectable after a longer ageing period.

The recording that corresponds to 11 days of ageing in Figure 23 has a TEG peak that is reduced in intensity compared to day 0 (compare to the intensity of naphthalene). This illustrates that the ageing process consumes TEG. Formates are also produced since tetraethylene glycol monoformate is found at 10.75 min and tetraethylene glycol diformate at 11.33 min in the chromatograms corresponding to 11 and 14 days of ageing. Weak signals corresponding to triethylene glycol, triethylene glycol monoformate and triethylene glycol diformate are found after 7.63 min, 8.37 min and 9.05 min respectively after 11 and 14 days. Diethylene glycol and diethylene glycol monoformate have very small peaks after 5.07 min, 5.58 min. respectively. Only the peak that corresponds to TEG has been calibrated quantitatively. The smaller, more volatile products appear in what looks like relatively small quantities in the chromatograms because they are more likely to evaporate from the reaction vial. The formation of esters in the reaction is supported by information from ATR FT-IR spectra as shown in Figure 24. Here, it is seen that the

most prominent difference between fresh TEG and TEG that has been aged for 36 days is an intense peak at 1717 cm^{-1}. The carbonyl group of carboxylic acids normally has a C=O stretch vibration in the 1725 cm^{-1} - 1700 cm^{-1} region and for esters this vibration is normally in the 1750 cm^{-1} - 1730 cm^{-1} region.[75] Carboxylic acid also has an OH stretch band in the 2400 cm^{-1} - 3400 cm^{-1} region. The spectrum of aged TEG has an OH stretch band but so does fresh TEG (from the end groups). Judging from the width of the carbonyl peak in the degraded TEG and the little shoulder on it, there could be more kinds of carbonyl compounds in this liquid.

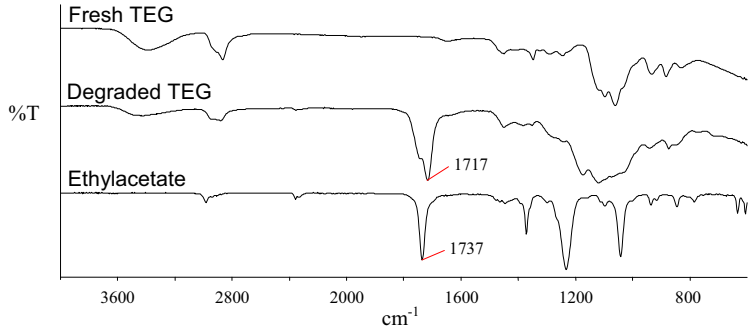


Figure 24. ATR FT-IR recordings of pure TEG, of TEG that has been degraded for 36 days in the reaction vial. Also shown is the spectrum of ethylacetate for reference.

When compared to an ester like ethylacetate as shown in Figure 24, it is seen that the ester functional group is a reasonable suggestion for the identity of at least one of the components in the mixture. Thus the ATR FT-IR spectra are in agreement with the formation of esters upon accelerated ageing of TEG.

The condensates collected in the condenser vials at –10 °C were also analysed. The GC-MS recording shown in Figure 25 corresponds to a fraction collected from the condenser vial after 38 days of ageing of TEG under air. The chromatogram has some overlapping peaks, so tracing characteristic *m*/*z* values helps in the assignment. The internal standard, naphathalene, is seen at 9.92 min in the TIC chromatogram.

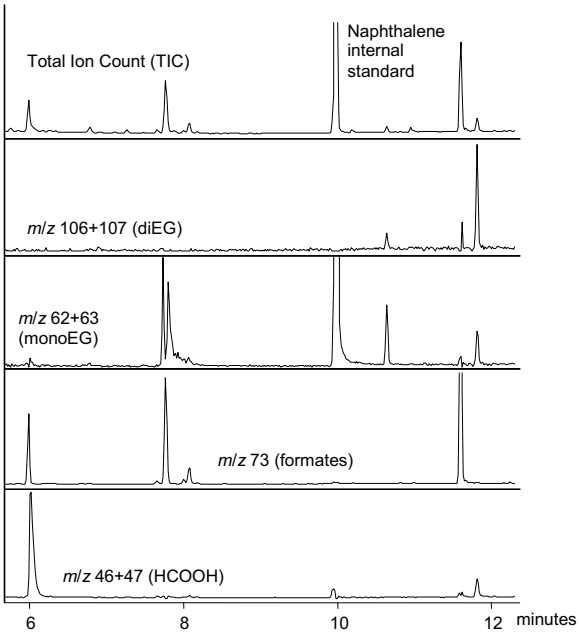


Figure 25. GC-MS recordings of condensates collected after 38 days of ageing of pure TEG in air. Degradation products seen here (relatively volatile) are mono and diethylene glycol (*mono*EG and *di*EG) and the mono- (*mono*EG-F and *di*EG-F) and diformates thereof (*mono*EG-F_2 and *di*EG-F_2) and formic acid. Five different traces are shown for the same gas chromatogram; TIC, *m*/*z* 106 and 107, *m*/*z* 62 and 63, *m*/*z* 73 and *m*/*z* 46 and 47.

The mass spectrometer used to characterize the condensates has a strong tendency to generate protonated molecular ions. This results in a peak, in the mass spectrum of ethylene glycol, at *m*/*z* 107 corresponding to protonated diethylene glycol (MH+). The trace of *m*/*z* 106 + *m*/*z* 107 is shown in Figure 25 which also reveals that diethylene glycol has retention time 11.78 min. For monoethylene glycol the MH+ is at *m*/*z* 63, the *m*/*z* 62 + *m*/*z* 63 trace shows that monoethylene glycol appears at retention time 7.71 min. *m*/*z* 73 always dominate in recordings of formates both mono and diformates, as confirmed by reference material. Monoethylene glycol monoformate, monoethylene glycol diformate, diethylene glycol monoformate and diethylene glycol diformate are found at retention times 5.97 min, 7.78 min, 11.64 min and 11.56 min respectively. The monoethyleneglycol diformate is overlapping the monoethylene glycol peak. Formic acid is found at 5.88 min. This peak also contains monoethylene glycol monoformate. The presence of formic acid in this peak is confirmed by the trace of *m*/*z* 46 + 47 where m/z 47 corresponds to the

characteristic ion of protonated formic acid. The retention time is also in agreement with formic acid reference chromatograms.

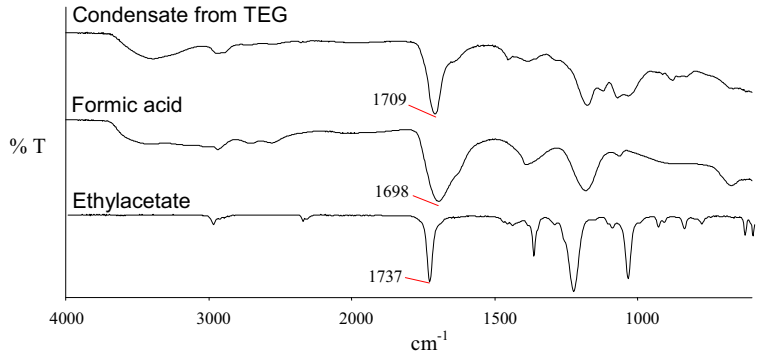


Figure 26. ATR FT-IR recording of condensate collected over 36 days of accelerated ageing of pure TEG. Also shown are the spectra of formic acid and ethylacetate.

In the infrared spectra recorded on condensates of TEG aged under air for 36 days the carbonyl peak is found at 1709 cm^{-1} (Figure 26). Formic acid and ethylacetate spectra are shown for comparison, the carbonyl peak of the spectrum of the condensate could represent both esters and carboxylic acids.

3.3.2 Accelerated ageing of PEG 1500

PEG 1500 was aged at 90 °C using the setup described in Article II. This was carried out by Egsgaard H., using electrospray ionization (ESI) mass spectrometry to characterize the degradation products.[76] After bubbling dry air through the molten PEG 1500 overnight, the ESI MS spectrum shown in Figure 27 was recorded. Dominant signals are observed due to $[PEG\ NH_4]^+$ adduct ions which correspond to PEG_{27}, PEG_{28} and PEG_{29} (PEG containing 27, 28 and 29 monomeric units, respectively). The spacing of one mass unit in the isotopic pattern reveals that the ions are singly charged. The two signals in between the PEG signals are interesting. They are located 14 and 28 mass units over the parent PEG signal.

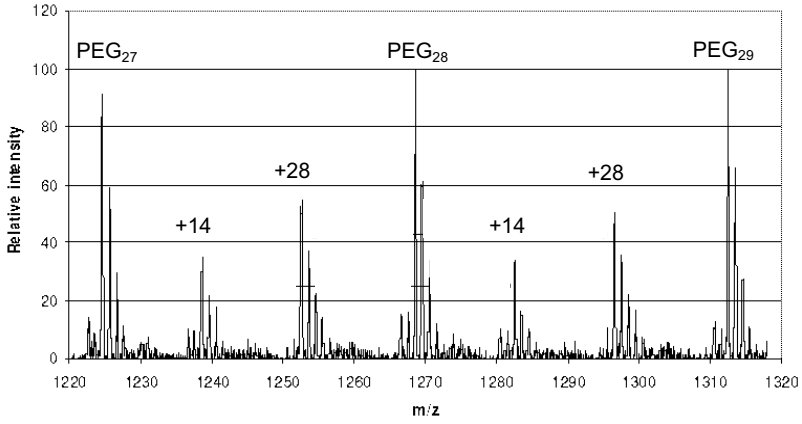


Figure 27. ESI mass spectrum of PEG 1500 degraded overnight at 90 °C with air bubbling through the liquid, recorded by Egsgaard H.[76]

When the aged PEG 1500 solution was hydrolysed by the addition of water, both the +14 and +28 peaks disappeared. Thus, the peaks correspond to products prone to hydrolysis. Assignment of *m*/*z* +14 satellite ions in MALDI-TOF MS was discussed in Chapter 2. In the present experiment ESI MS is used so salt clusters can hardly form and the only hydrolysable species that can be assigned to the *m*/*z* +14 ion is an internal ester. The signal at *m*/*z* +28 also disappeared after hydrolysis. Thus a formate of PEG seems to be a good assignment.

To look further into this a PEG formate was prepared by reacting fresh PEG 1500 with a mixture of formic acid and acetic anhydride. ESI spectra of this reaction mixture had abundant *m*/*z* +28 ions but no *m*/*z* +14. When performing a CID (collision induced dissociation) experiment on the +28 ion in the PEG formate, a characteristic loss of *m*/*z* 28 is observed and it can be assigned to loss of CO from the formate moiety. The same behavior was observed for the *m*/*z* +28 ion in the aged PEG 1500 solution. A unique assignment for the +14 was not possible. All in all this experiment suggests that thermo-oxidation of PEG 1500 leads to the formation of formates of the PEG molecules and that internal esters are produced too.

3.3.3 Effect of additives on rate of degradation of TEG

It was shown in the article that the weight-loss of the reaction vials could be used as a measure of TEG-degradation. In Figure 28 the weight-loss is shown for six reaction vials over 518 days. Vial A contains TEG without any additives and dry air bubbles through the liquid, while in vial B nitrogen

bubbles through TEG. It is seen that vial A weighs approximately one fourth of its initial weight after 52 days whereas vial B which is under nitrogen loses weight very slowly. This vial (B) was dropped at day 71, which is why the mass drops so suddenly. The analogous curves with iron(III)sulfate added in catalytic amounts have the same shape. Under air the TEG is degraded after about 50 days, under nitrogen the weight-loss is moderate and equals 38 % of the initial weight after 518 days. This shows that iron(III)sulfate has no effect on the TEG-degradation. The vials containing potassium iodide showed much better stability towards thermo-oxidation. Vial C had KI added and was aged under air. Vial D had KI added and was aged under nitrogen. It is seen that vial C lost weight almost as slowly as the vials aged under nitrogen. This demonstrates that potassium iodide inhibits thermo-oxidation of TEG at 70 °C very well. The moderate weight-loss over 500 days in the vials that had nitrogen bubbled through (vials B, D and F) must be due to either evaporation of TEG or simply loss of substance of the vial through handling when weighing them. The Teflon tubes were removed from the vials every time they were weighed and even though this was done very carefully the vials were weighed 33 times over the entire period of ageing. A final possibility is pyrolysis of the TEG aged under nitrogen. All three possibilities may have contributed over the extended period of ageing shown in Figure 28.

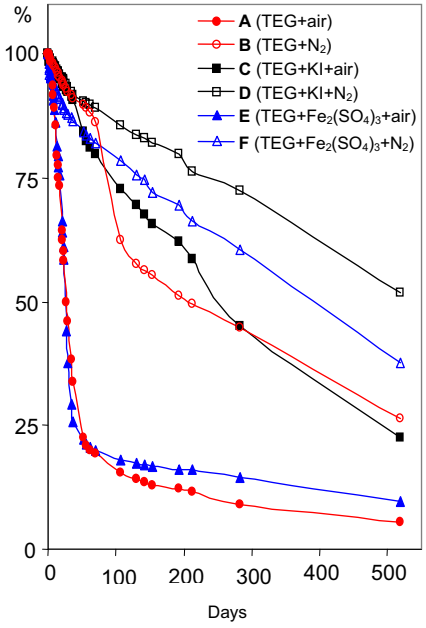


Figure 28. Mass of the contents of the reaction vials as a percentage of the initial mass (weight-loss) is plotted versus days of ageing. Circles: pure TEG (closed: air, open: nitrogen). Squares: TEG with ca. 10 mM potassium iodide (closed: air, open: nitrogen). Triangles: TEG with ca. 10 mM iron(III)sulfate (closed: air, open: nitrogen). Pure TEG and TEG containing iron(III)sulfate lose weight in air, but TEG with potassium iodide does not. Under nitrogen, only a small weight-loss is observed.

The experiment in Figure 28 demonstrates an extremely persistent antioxidant effect of potassium iodide on thermo-oxidation of TEG at 70 °C. Only 10 mM KI was added to the TEG, which results

in a ratio of 580 TEG molecules per KI. This ratio in combination with the long timespan that the KI works on, suggests that the antioxidant effect must be catalytic because KI would have been used up far earlier than 518 days if it had been a stoichiometric reaction between oxygen in the air and KI.

As discussed in Article II, a large group of components was screened to see how each changed the rate of TEG-degradation. The results are illustrated in Figure 29 where a straight line is drawn through the three points corresponding to the three times the vials were weighed. When the results are viewed this way, it is easy to see that a group of vials (black lines in Figure 29) lose weight at approximately the same rate as a vial containing TEG without any additives (red line in Figure 29).

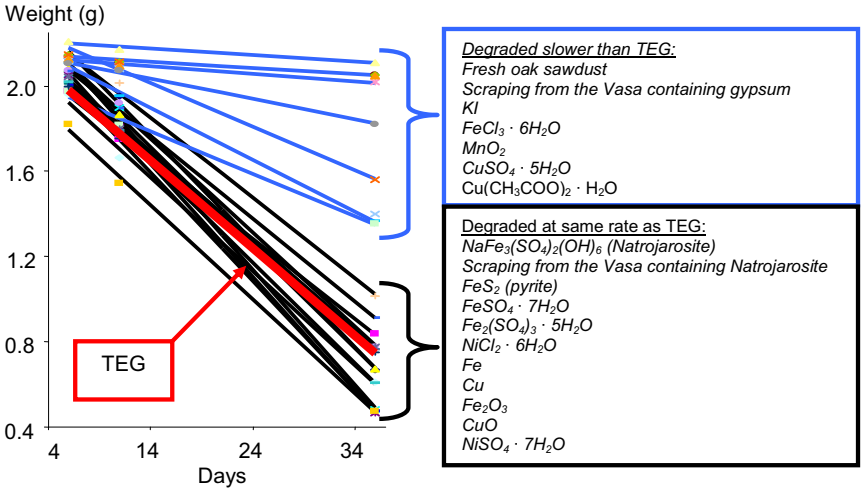


Figure 29. Mass (g) of the contents of reaction vials as function of days of ageing is shown for 20 reaction vials (left). Small quantities of the compounds listed in the two boxes (right) were added to reaction vials containing TEG. Red line: pure TEG, black lines: group that loses weight at rates similar to pure TEG, blue lines: group that loses weight more slowly than pure TEG.

This is an indication that the compounds added to these vials have very little or no effect on the degradation of TEG.

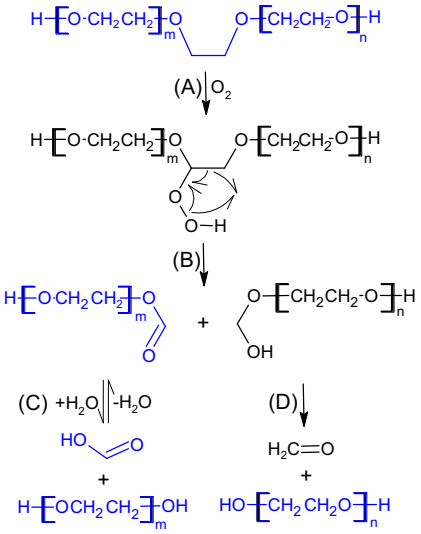
Another group of compounds (blue lines in Figure 29) had an effect on the weight-loss of the vials. This group of compounds includes potassium iodide and other components that slow down the weight-loss. The criterion used to distinguish between stabilised and non-stabilised vials was that the slope, which was 0.04 g/day for TEG, should deviate more than plus or minus 0.01 g/day from this, i.e. slopes of 0.03 g/day or lower are taken to be stabilised. The intention with this experiment is not to find accurate kinetic information about the salts in the reactions (three measurements in one curve is not enough for this) but merely to perform a coarse screening of a large number of compounds for effect on TEG-degradation. Furthermore, the duration of one month for this experiment may not be enough to say if a reaction is catalytic or stoichiometric. For example, the vial with sawdust had a relatively large amount of added sawdust (see Article II) and it seems that

the protective effect of the wood persists for the duration of this experiment even though the antioxidative effect is probably stoichiometric (discussed later).

3.4 Mechanism

3.4.1 PEG degradation mechanisms

The observations in the accelerated ageing experiments were interpreted in terms of a TEG-degradation mechanism in Article II, which is also shown in Scheme 2. This mechanism is not necessarily the only mechanism in the degradation of TEG. It is normal to have a number of processes involving radicals (alkyl ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$)) and hydroperoxides (ROOH) in a room temperature reaction between oxygen and a polymer. Such a room temperature oxidation is commonly known as an autoxidation with cross-linking between polymers and chain scissions as the results.[77] The reaction discussed here merely involves a hydroperoxide, but it accounts for the products observed in the experiment. These products were formic acid, mono, di- and tri- ethylene glycol and mono- and diformates of mono-, di-, tri- and tetraethylene glycol. In the mechanism shown in Scheme 2, the species in blue have been detected with GC-MS.



Scheme 2. Proposed degradation mechanism for tetraethylene glycol (for TEG m+n+1=4). The species in blue were detected by GC-MS. A hydroperoxide is formed on TEG in reaction A, for an in-chain hydroperoxide both m≠0 and n≠0. Rearrangement of the hydroperoxide (reaction B) leads to a formate and a hemiacetal. The formate is in equilibrium with the alcohol and formic acid (reaction C) via esterification/hydrolysis. The unstable hemiacetal breaks down to formaldehyde and the alcohol in reaction D. A hydroperoxide situated at a terminal monomeric unit would produce either formic acid (m=0) or methanediol (n=0) depending on the position relative to the hydroxo group. Methanediol would dehydrate to give formaldehyde (reaction D, n=0).

In the proposed degradation mechanism tetraethylene glycol (m+n+1=4) is degraded but the mechanism should be valid for larger PEG molecules too. In the first step it is suggested that a hydroperoxide is formed (reaction A Scheme 2) on the TEG (or the polymer). If this is situated in-chain (both m≠0 and n≠0) the rearrangement shown in reaction B Scheme 2 will lead to a formate and a hemiacetal. The hemiacetal is unstable and it will rearrange to give formaldehyde and an alcohol (Scheme 2, reaction D). The formate is in equilibrium with the alcohol and formic acid (Scheme 2, reaction C) via esterification/hydrolysis. For a terminal hydroperoxide, either formic acid (m=0) or methanediol (n=0) would be produced depending on the position in the terminal monomeric unit of the hydroperoxide. If methanediol is formed, it will dehydrate quickly under the circumstances of the experiment (dry air at 70 °C), leading to formaldehyde and water (Scheme 2, reaction D, n=0).

No formaldehyde was observed in the reaction vials or condenser vials used for the ageing of TEG. The boiling point for formaldehyde is –19 °C, which means that it would be impossible to trap formaldehyde itself in the condenser vials at –10 °C. However, formaldehyde readily forms trimers as illustrated in Figure 30 for the formation of 1, 3, 5-trioxane. The boiling point of trioxane is higher than that of formaldehyde.

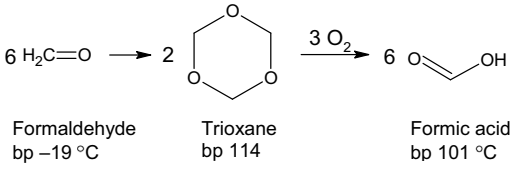


Figure 30. Formaldehyde produced in the degradation of TEG would evaporate from the reaction vial at 70 °C. It can also form 1,3,5-trioxane as shown. This species might oxidise to formic acid before it evaporates from the reaction vial at 70 °C.

It seems realistic that this species can stay in the reaction vial at 70 °C without evaporating for long enough to get oxidised to formic acid.

It was mentioned that the degradation of TEG or PEG, as described in Scheme 2, could be accompanied by other reactions even though all products were explained by the reaction in Scheme 2. The first step in the reaction was the formation of a hydroperoxide. Here, it is likely that C-H bond homolysis in TEG precedes the reaction with dioxygen giving the hydroperoxide. This would imply that free radicals are in the solution after homolysis. If the presence of a hydroperoxide is accepted, then accepting the formation of hydroxyl ($HO^{\bullet}$) and alkoxyl radicals ($RO^{\bullet}$) is not far away since the O-O bond is generally weak in hydroperoxides. These ideas are all supported by the fact that phenol antioxidants slow down thermo-oxidation of PEG, which has been demonstrated in the literature.[27,67] The action of phenol antioxidants is to remove radicals as explained in Figure 20 and Figure 21 not to react with hydroperoxides. This suggests that, not only hydroperoxides, but also free radicals play a role in the degradation.

Heatly and co-workers have proposed a mechanism that is practically identical with the one discussed here in Scheme 2. The only difference is that they suggest that the breakdown of PEG is

by Random Chain Scission (RCS) in experiments focused on high molecular weight PEG.[37-39] Their reaction involves the same functional groups as suggested here in Scheme 2. Glastrup has suggested that the reaction may be end degradation in an experiment with triethylene glycol where the end groups had been modified (described in the introduction).[42] The functional groups involved in this mechanism[41] are also accounted for in the mechanism given here (Scheme 2). In most of the work on polymer degradation, hydroperoxides and radicals are central points in the explanation of the degradation. It is assumed that hydroperoxide formation plays an important role in the present work too, formation of a hydroperoxide of TEG is taken to be the initial reaction as illustrated in Scheme 2A. It is also assumed that the formation of a hydroperoxide probably requires homolysis of the C-H bond before oxygen can be inserted between C and H in TEG (reaction A, Scheme 2). Thus the relative stabilities of the radicals generated on the polymer chain affect the possible regioselectivity of the hydroperoxide in PEG. This is illustrated in Figure 31.

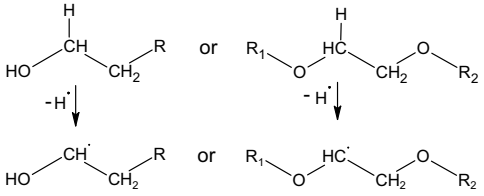


Figure 31. The radicals generated in PEG upon hydrogen atom abstraction in end degradation and in-chain reaction. R, R_1, R_2 represents the remaining parts of the polymer (-[CH_2CH_2O-]$_n$-H).

When comparing these possibilities, radical hyperconjugation does not seem to favour end-degradation. In both cases the radical is on a carbon that has a methyl group and an oxygen atom as the nearest neighbours. If one possibility should be energetically favoured over the other it would be the in-chain radical because it has a long polymer chain (R_1 and R_2 in Figure 31) on both sides of the radical to stabilize it, the terminal radical has a polymer chain only on one side. This points to an in-chain reaction or at least a random chain scission (RCS) rather than an end-degradation. Clearly molecular weight has a lot to say in the matter. If the C-H bond energies were equal in the entire polymer, the probability for in-chain degradation in tetraethylene glycol would be 0.5 ((DP-end groups)/DP) but 0.98 for PEG 4000 (DP=91, DP: Degree of Polymerisation). In other words, if the end degradation and in-chain degradation should take place an equal number of times in PEG 4000, the reactivity would have to be 49 times higher for the end groups than for in-chain groups (0.98/0.02). Although the mechanism suggested in Scheme 2 accounts for products formed by both end degradation and by in-chain reaction, it seems likely that large PEG molecules mostly take the in-chain route.

An observation is described in the literature:[17,41,73] degradation is faster when dry air is bubbled through the liquid than when moist air is bubbled through. It was shown by Glastrup[41] that thermo-oxidation of TEG at a relative air humidity of 75 % leads to formic acid and shortened oligoethylene glycols but no esters of either of the ethylene glycols were detected at any time in the degradation. An interpretation of this information may be possible within the principles of hydrogen atom abstraction and hydroperoxide formation used to explain the mechanism under dry air (Scheme 2). It can be concluded that formates were produced in the reaction under dry air, at least to an extent by esterification with formic acid, since formates of tetraethylene glycol are seen early in this reaction. Formates that are produced from the degradation directly (as in Scheme 2 reaction B) would not be seen on TEG only on triethylene glycol and lower. Thus there is an equilibrium

between formic acid and esters that is controlled by water as shown in Scheme 2. When the air is humid, water is present and esters hydrolyse, the degradation is reported to be slower in this situation.

In the absence of moisture, formates are formed and the degradation is fast. This suggests that formates de-stabilise TEG. A possible explanation is given in Figure 32. The bond dissociation energy might be lower for the hydrogen on the carbonyl carbon of the formate than it is in-chain. For example the bond dissociation energies ($D°_{298}$) for **H**-CH_3, **H**-CH_2OH, **H**-CHO and **H**-$COOCH_3$ are 439 kJ/mol, 402 kJ/mol, 369 kJ/mol and 388 kJ/mol respectively.[78] This suggests that it is easier to abstract a hydrogen atom from the carbonyl carbon of the formate rather than in-chain. This is shown in reaction A in Figure 32. Carbon dioxide, a very stable product, is cleaved off from the radical formed (B) and an alkyl radical is left. It reacts with oxygen (C) to produce a peroxy radical that can abstract a hydrogen atom (D) to form a hydroperoxide. This hydroperoxide can then enter the degradation described in Scheme 2.

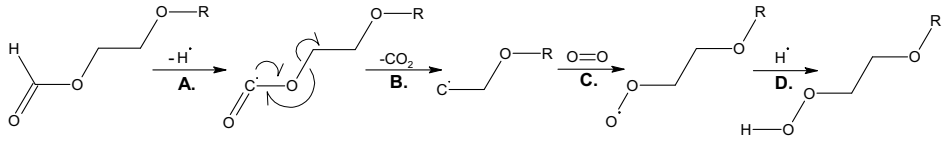


Figure 32. Possible reaction of PEG formates. A hydrogen atom is abstracted from a formate (A). The formed radical rearranges to yield carbon dioxide and an alkyl radical (B). The alkyl radical may react with oxygen to produce a peroxy radical (C) that may abstract a hydrogen atom to produce a hydroperoxide that can enter the degradation mechanism described in Scheme 2. R represents the remaining part of the polymer (-[CH_2CH_2O-]$_n$-H).

In this mechanism formates can initiate degradation and since they are situated at the end groups, where the alcohols are in PEG, degradation is initiated here. If the hydrogen atom at the formate really is more labile than the hydrogen atoms in-chain, then end-degradation would be a little more favourable under dry conditions. However, this reaction can be no more than an additional route to the one described in Scheme 2. This is because the formic acid in the formate end groups has to come from the reaction described in Scheme 2 in order for the reaction in Figure 32 to be able to run.

Formates and in-chain esters were discussed as possible assignments to the satellite peaks observed at +14 and +28 in the ageing of PEG 1500 (Figure 27). The in-chain esters and carboxylic acids are not accounted for in the proposed mechanism in Scheme 2. However, it is not a big step from the proposed mechanism to something that can accommodate in-chain esters, too. If an in-chain hydroperoxide is formed in reaction A Scheme 2 one can easily imagine that a C-H bond is broken instead of the C-C bond that is broken in Scheme 2B. This would lead to dehydration as shown in Figure 33.

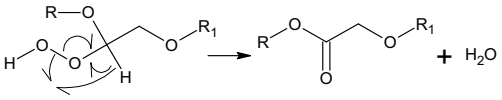


Figure 33. Formation of in-chain esters by dehydration of a hydroperoxide. R and R_1 represent the remaining part of the polymer (-[CH_2CH_2O-]$_n$-H).

This reaction was also discussed by Morlat and Gardette[54] in the introduction to this chapter.

It is evident that the PEG degradation mechanism suggested here is just one out of many mechanisms proposed in the literature. Most of these mechanisms involve some combination of hydroperoxides and radical chemistry. The mechanism considered here only deals with hydroperoxides. However, when hydroperoxides are present it is not unlikely that alkyl, alkoxyl or peroxyl radicals are also present because they can form from the hydroperoxide. It was also explained in the introduction that pyrolysis can take place at low temperatures (150 °C) and it may not be unlikely that such reactions take place at 70 °C too, in the background. Clearly many reactions are possible when large molecules such as PEG are exposed to radicals because they are normally reactive species with low selectivity. However under the circumstances of these experiments it seems that the main product of degradation is formic acid and that can be explained by the mechanism discussed in Scheme 2.

3.4.2 Antioxidant mechanism

The fact that TEG-degradation was inhibited by a large number of very different compounds in what seemed like a catalytic antioxidant reaction has been puzzling for a long time and still is. Several explanations have been considered, including some that involve the removal of the initial hydroperoxide. If it is assumed that degradation is driven by hydroperoxide O-O bond homolysis, then removal of the hydroperoxide will lead to inhibition of the degradation. Lowering the hydroperoxide concentration lowers the probability of formation of alkoxyl $RO^{\bullet}$ and hydroxyl $HO^{\bullet}$ radicals that are even more damaging to the polymer. Clearly, removal of any of the radicals (alkyl $R^{\bullet}$, alkoxyl $RO^{\bullet}$, hydroxyl $HO^{\bullet}$, peroxyl $ROO^{\bullet}$) and of hydroperoxides (ROOH) would serve to protect the polymer. Hydroperoxide removal is just one way of slowing down degradation.

One example of a mechanism from the literature is the metal ion-hydroperoxide complex formation discussed by Lloyd.[74] Here it is the idea that the metal ion promotes the formation of a six-membered intermediate where the hydroperoxide was removed by a heterolytic process that also involves a hydroxo end-group (Figure 34). The rearrangement yields formaldehyde water and a formate of PEG.

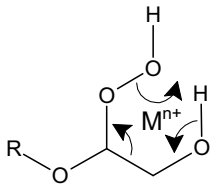
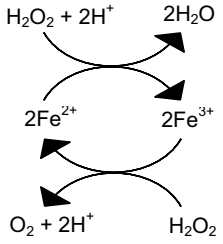


Figure 34. Metal ion – hydroperoxide coordination as discussed by Lloyd.[74] The hydroperoxide reacts with the hydroxo end group to form formaldehyde, water and a formate of PEG. R represents the remaining part of the polymer, M^{n+} is the metal ion.

One of the qualities of this mechanism is that it leaves the metal unchanged after the reaction, thus the metal is not consumed. A large group of different salts would probably also be able to form the complex in accordance with the observations made in the present experiment. A drawback is that rearrangement is believed to work only for hydroperoxides positioned at the second carbon from the terminal alcohol. This is because the terminal hydroxo group is believed to be crucial in the coordination of the metal ion, suggesting that the inhibition only works at the end groups of the polymer. Another feature is that the reaction consumes polymer and produces degradation products like the ones formed in Scheme 2. Thus in order to remove one hydroperoxide, one polymer molecule is sacrificed, according to Figure 34.

Another interesting explanation was considered for a while in our group. If the TEG hydroperoxide is compared to hydrogen peroxide, then some interesting analogies emerge. It is known that potassium iodide, manganese(IV)oxide and many other compounds catalyse the disproportionation of hydrogen peroxide to give oxygen and water. This is a well-known problem in the manufacture of hydrogen peroxide where precautions are taken to avoid contaminating the product with ions that can catalyze its degradation.



Redox pairs	E° (SHE)
$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$	1.76 V
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	-1.03 V
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77 V
$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$	1.23 V
$I_2 + 2e^- \rightarrow 2I^-$	0.535 V
$HIO + H^+ + 2e^- \rightarrow I^- + H_2O$	0.987 V
$Cu^{2+} + e^- \rightarrow Cu^+$	0.159 V

Figure 35. Catalytic cycle for iron in the disproportionation of hydrogen peroxide.[79] Standard redox potentials (E°) are given in the box for half-reactions in acidic solution.[78]

In Figure 35, the generally accepted catalytic cycle is shown for iron catalyzing the disproportionation of hydrogen peroxide.[79] The idea behind this is that iron shuttles back and forth between two oxidation states transporting electrons in the process. In the first part of the cycle (top) hydrogen peroxide reacts with two protons to form water. The two electrons that go into this reaction are provided by oxidation of two iron(II)ions. The two iron(III)ions produced are reduced back to iron(II)ions in the reaction with hydrogen peroxide (bottom) giving oxygen and the two protons back. In order for an ion to be suitable as a catalyst for disproportionation of hydrogen peroxide, it must be able to shuttle between two oxidation states and the redox potential must be between 1.76 V and –1.03 V vs. SHE. These limits correspond to the redox potentials for the two half-reactions in the disproportionation* shown in the top of the box in Figure 35. As is also seen in the box, a whole range of half-reactions relevant to the present study can be listed. They include reactions involving iodide, manganese, copper and iron. Many more could have been listed that all live up to the redox potential requirement.[78] A large number of the salts that catalyze disproportionation of hydrogen peroxide also inhibit TEG-degradation. If the initial TEG hydroperoxide was removed catalytically by the mentioned ions, then the catalytic antioxidant effect would be explained. If half-reactions for the hydroperoxide are written analogous to the hydrogen peroxide half-reactions, then it is seen that TEG is consumed and new products are formed in addition to oxygen and water.† These new products would either be an ether between two TEG molecules or a TEG hemiacetal leading to an aldehyde and an alcohol. It is realized that this mechanism is speculative but it is fascinating and it is not in the literature.

The antioxidant mechanisms discussed here are interesting but a lot of work would remain before the phenomenon can be explained in detail. However, it is clear that the salts did slow down the degradation of TEG and it is believed that the removal of hydroperoxide is responsible somehow.

3.4.3 Relation to PEG-treated wood

It was shown in the present work that TEG degrades readily at 70 °C and PEG 1500 at 90 °C under air. There is nothing that suggests that degradation cannot take place at temperatures lower

* For the catalyst to be able to cycle the following half-reactions must be endergonic ($E^\circ > 0$):

$H_2O_2 + 2H^+ + M^{red} \rightarrow 2H_2O + M^{ox}$ $\quad E_{tot}^\circ = E^\circ_{water} - E^\circ_{ion} > 0 \Rightarrow 1.76\ V > E^\circ_{ion}$

$H_2O_2 + M^{ox} \rightarrow O_2 + 2H^+ + M^{red}$ $\quad E_{tot}^\circ = E^\circ_{ion} - E^\circ_{oxygen} > 0 \Rightarrow E^\circ_{ion} > -1.03\ V$

† The half-reactions of the disproportionation of TEG hydroperoxide written analogously to the hydrogen peroxide reactions:

TEG-OOH + TEG^+ + $H^+ \rightarrow$ 2 TEG-OH (TEG-O-TEG and H_2O are also possible products)

TEG-OOH $\rightarrow$ $TEG^+ + H^+ + O_2$

than this. However, this does not necessarily mean that the reaction is ongoing in exhibited wooden museum objects. It was seen that formic acid is the primary degradation product of both PEG and TEG. If PEG degradation takes place or has taken place in PEG-impregnated objects, then formic acid should have formed assuming the reaction mechanism is the same at room temperature as it is at the experimental conditions. Thus elevated amounts of formic acid in PEG treated museum objects could be an indication that PEG degradation has taken place.

It was seen in the experiments with TEG and additives that many different compounds affect the rate of degradation of the TEG. Some of those compounds are relevant to PEG-treated shipwrecks directly. As was also discussed in Article II, some salts with no effect on the degradation relate to salts detected in PEG-treated shipwrecks such as the Batavia (αFeO(OH), FeO(OH), $Fe(OH)_2$, S, FeS_2, $FeSO_4{\cdot}4H_2O$, $FeSO_4{\cdot}5H_2O$, $NaFe_3(SO_4)_2(OH)_6$, $KFe_3(SO_4)_2(OH)_6$, $Fe_3(SO_4)_4{\cdot}22H_2O$, $FeSO_4(OH){\cdot}2H_2O$ and $Fe_3(SO_4)_4{\cdot}14H_2O$),[80] the Mary Rose ($FeS_2$, Fe_8S_9, $FeSO_4{\cdot}4H_2O$, $FeSO_4{\cdot}7H_2O$ and $NaFe_3(SO_4)_2(OH)_6$),[81] the Vasa ($CaSO_4{\cdot}2H_2O$, $NaFe_3(SO_4)_2(OH)_6$, $FeSO_4{\cdot}7H_2O$ and S_8),[82,83] and on the Skuldelev ships around the nail holes ($FeSO_4{\cdot}4H_2O$).[2] This shows that some of the components that are in PEG-treated shipwrecks naturally have no influence on PEG degradation.

In the group of components that had an antioxidant effect in this experiment, two are definitely present in the Vasa, namely the oak wood sawdust and the scraping from the Vasa containing gypsum. Some of the other compounds that were effective antioxidants may or may not be in the Vasa or in other waterlogged wooden objects - this is not yet known. Whether the effect of the scraping was due to gypsum or perhaps to wood dust in the sample is hard to say, but it is interesting to realize that some components of the Vasa retard degradation of TEG. This might suggest that TEG, or PEG in the case of the Vasa, only degrades after the wood has reacted with the oxidant. Theoretically there is also the option that components in the wood inhibit the degradation of PEG without reacting themselves. As was discussed in Article II, wood and plant components have been found to act as antioxidants. For example Mikhal´chuk *et al*.[70] found that several plant phenols (herb extracts containing caffeic acid, syringic acid and phloroglucinol) inhibit thermo-oxidation of PEG at 80 °C. Other experiments confirm that wood contains components with antioxidant properties.[84-86] Han *et al*.[67] found that polyphenole antioxidants, protect PEG 6000 from oxidation at 80 °C. Maybe this is not so surprising if one compares the structure and reactions of the commercial hindered phenol antioxidants (Figure 20 and Figure 21) with the structure and polymerization of monolignols in Figure 4 and Figure 5. It could be speculated that free radicals or oxidative stress have a positive effect on the mechanical properties of old, or de-polymerized lignin because they could lead to re-polymerisation as in Figure 4. The wood itself would have to work in a way that is similar to the way phenol antioxidants work. As seen in the introduction (Figure 4 and Figure 5), this is a stoichiometric reaction which means that the wood is consumed in the process. Thus the antioxidative properties would wear out when all the available phenols had reacted. It can be concluded that wood inhibits thermo-oxidation of TEG under the conditions of the accelerated ageing described. It is very tempting to apply this conclusion to PEG-treated shipwrecks, but there are many differences between the experiment and the situation in the timbers of a PEG-treated shipwreck. The ageing experiment was done at 70 °C with a steady flow of air into the liquid medium. The situation in PEG-impregnated wood is different: room temperature, slow air supply, solid medium meaning that the antioxidant properties are not necessarily available to the PEG in the same way as in the ageing experiment.

3.5 Further conclusions

It was demonstrated that formic acid is the main product of thermo-oxidative degradation of TEG. Formic acid is also expected to be present in objects where PEG degradation has taken place.

PEG 1500 undergoes a similar reaction to that seen in TEG, which leads to the formation of satellite peaks at *m*/*z* +14 and *m*/*z* +28 in ESI MS. The signal at *m*/*z* +28 was assigned to formates of PEG. This assignment was confirmed by CID. In-chain esters were proposed for the signal at *m*/*z* +14. This is in agreement with the formation of in-chain hydroperoxides, as discussed above.

Catalytic amounts of potassium iodide protected TEG against thermo-oxidation at 70 °C. After 518 days, there was still TEG left. Potassium iodide was not the only compound that slowed down the thermo-oxidation of TEG. Oak sawdust, scrapings from the Vasa containing gypsum, iron(III)chloride, manganese(IV)oxide, copper(II)sulfate and copper(II)acetate also had an antioxidant effect.

Oak wood sawdust and a scraping from the Vasa slowed down TEG-degradation. It was speculated that phenolic fragments of lignin in the wood react stoichiometrically with radical species in a way that resemble polymerisation of monolignols or the action of commercially known phenol antioxidants.

4 Formic acid as a marker for PEG degradation

One of the main goals in this project is to find out if PEG degradation is taking place or has taken place in the Vasa timbers and in PEG-treated archaeological wood in general. It was established in Chapter 3 and in Glastrup[41] that formic acid is a key product of TEG and PEG degradation. This means that if PEG degradation has taken place in a PEG-impregnated shipwreck such as the Vasa then formic acid has been produced. One could imagine that formic acid is in archaeological wood naturally, which would make it more difficult to use formic acid as marker for PEG degradation. Nevertheless the work in the present chapter deals with formic acid as a marker molecule for PEG-degradation. The formic acid contents as described by Glastrup *et al*.[87] were discussed and investigations were carried out to determine the origin of the formic acid, whether it originated from PEG or from wood. Several techniques had to be developed to get this information. Thus the present chapter deals with development and validation of these methods to a large extent as well as with formic acid content and origin. The methods include; a technique for measuring formic acid concentrations, for determining $H^{12}COO^-/D^{13}COO^-$ and $^{12/13}CO_2$ ratios and for isolating formic acid from wood. The first two methods were used for validating the third, the formic acid isolation method.

Information about formic acid in archaeological wood is scarce in the literature. However modern wood has been studied regarding formic acid content. For example McDonald *et al*.[88] found between 4 and 174 mg formic acid per m^3 of air, when sampled from a kiln while drying fresh timber at 140 °C. Sundqvist *et al*.[89] finds between 1.1 % and 7.2 % formic acid in wood that has been treated hydrothermally at 180 °C for 4 hours. Even though these temperatures are not comparable to the temperature in a museum, these studies demonstrate that formic acid can form from wood under certain conditions. In the present chapter formic acid concentrations in PEG-treated archaeological wood as published by Glastrup *et al*.[87] are discussed.

Since formic acid is likely to be in wood naturally, it would seem that the formic acid content of a PEG-treated archaeological wood sample only gives information about PEG degradation if the source of the formic acid is well established. In the present work, this is done by measuring the ^{14}C content of formic acid isolated from a sample from the Vasa. If this ^{14}C content is the same as the ^{14}C content of the wood it was taken from, then the formic acid is a product of wood. On the other hand if the ^{14}C content is the same as in the PEG that the wood was impregnated with, then it is a product of PEG and thus indicates that PEG has degraded. This difference in ^{14}C content of PEG and wood from the Vasa is due to the fact that the Vasa is from 1628 and PEG is a petrochemical. A petrochemical is an oil product, which is ^{14}C-depleted, thus PEG is ^{14}C-depleted. The Vasa, on the other hand, sank in 1628 just after it had been built. The ^{14}C content of the wood in this ship should correspond to the years up to 1628 (96 pmC, percent modern Carbon). Therefore the ^{14}C content of formic acid reflects its origin: if formic acid in the Vasa is a product of PEG degradation, then it is ^{14}C-depleted. If it is a product of the Vasa wood it has a pmC of 96 like the wood. If both sources produce formic acid the ^{14}C content will be intermediate.

Formic acid will have to be isolated from the Vasa timbers, and a radiocarbon measurement done. In a radiocarbon analysis the content of carbon isotopes is analyzed by mass spectrometry. The naturally occurring carbon isotopes are ^{12}C, ^{13}C and ^{14}C, which are found in the ratio ^{12}C: ^{13}C: ^{14}C = 10^{12}: 10^{10}:1. ^{12}C and ^{13}C are stable, which means that they do not decay and thus their concentrations remain constant over time. ^{14}C is radioactive and decays with a half-life of 5730

years. It is a cosmogenic isotope which means that it is produced in the atmosphere by cosmic irradiation of, for instance, ^{14}N:

$$^{14}N + n \rightarrow {}^{14}C + p$$

Where n is a neutron and p is a proton. The ^{14}C formed is rapidly oxidised to $^{14}CO_2$ and enters the Earth's plant and animal life through photosynthesis and the food chain. When a plant dies it stops taking up new carbon, but the ^{14}C in it continues to decay by emitting a beta particle (β):

$$^{14}C \rightarrow {}^{14}N + \beta$$

Thus the ^{14}C content of plant material decreases from the time of death of the plant as illustrated in Figure 36A. The ^{14}C content of a sample can be translated back to the time of death of the plant, using the half-life of 5730 years for the ^{14}C isotope.

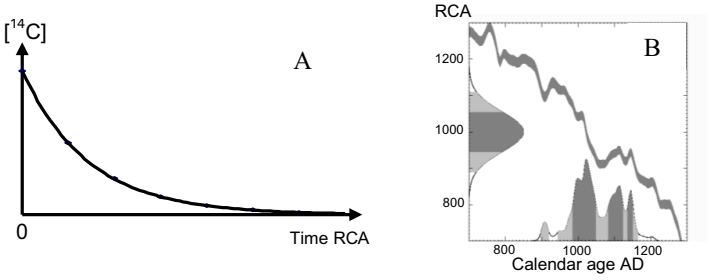


Figure 36. A: Decay of ^{14}C, the un-calibrated exponential decay relates to the so-called radiocarbon age (RCA) reported as years before present (BP) where 1950 is taken to be present. B: Calibration curve. The curve converts RCA into a calendar age. The curve is based on the dataset by Reimar *et al.*[90] using the computer software "Calib 5.0".[91]

A measurement of the ^{14}C concentration can be translated into a RadioCarbon Age (RCA). This number is given as the number of years before present (BP) where present refers to 1950 by convention. This RCA assumes that the content of ^{14}C in the atmosphere has been constant at all times. This is only partly true. Calibrations have been made by measuring the ^{14}C content in annual rings of old trees. An example is shown in Figure 36B where an RCA of 1000 BP is converted into a calendar age of 1020 AD. Following Stuiver and Polach conventions,[92] the RCA is calculated as follows:

$$RCA = -8033 \ln \frac{A_{SN}}{A_{ON}}$$

where A_{SN} is the activity of the sample (scintillation counting was used in the old days to find the activity of β decay which represents the ^{14}C content in the sample) and A_{ON} the activity of the international oxalic acid standard from 1950. The activities in this formula are both normalised (hence the N), that is they are corrected for isotopic fractionation using the measured $\delta^{13}C$ (per mil) as follows:

$$A_{SN} = A_S\left(1 - \frac{2(25 + \delta^{13}C)}{1000}\right) \qquad \text{and} \qquad A_{ON} = 0.95A_O\left(1 - \frac{2(19 + \delta^{13}C)}{1000}\right)$$

Isotopic fractionation is a subtle difference in reaction rate for isotopes. The lighter isotopes react slightly faster than the heavier ones. Thus $^{12}CO_2$ is taken up slightly faster by plant leaves than $^{13}CO_2$. This results in ^{12}C being slightly more abundant in plants than ^{13}C, an effect can be observed in all chemical and physical reactions involving more isotopes.

Another common way of representing ^{14}C content is the percent modern Carbon (pmC). It is given by the activity of the sample, A_{SN}, in percent of the absolute activity of the international oxalic acid standard, A_{ABS}.

$$pmC = \frac{A_{SN}}{A_{ABS}} 100$$

where A_{ABS} is A_{ON} corrected for date of measurement:

$$A_{ABS} = A_{ON} e^{\frac{y-1950}{82666}}$$

(all definitions according to Stuvier and Polach).[92] In the sections that follow, isolation and measurement by AMS (Accelerator Mass Spectrometry), of formic acid from PEG-impregnated archaeological wood is described. Preliminary AMS results are given for a sample from the Vasa.

4.1 Method for measuring formic acid concentration

If formic acid is a key product of PEG degradation as suggested, then the formic acid contents of a sample would be a marker of PEG degradation. For this purpose, a technique is required that is capable of measuring formic acid concentrations that are lower than 0.1 % by weight. SPME GC-MS (Solid Phase Micro Extraction Gas Chromatography-Mass Spectrometry) equipment enabled the development of a method that meets these requirements. In the present case, the SPME fiber allowed the analysis of formic acid in the headspace over a powdered wood sample dispersed in a sulphuric acid solution. The sulphuric acid acts by releasing formic acid from the wood and into the gas phase. The technique for measurements on wood samples is described in Glastrup *et al.*[87] The following section describes the calibration of this method for analysis of formic acid in aqueous solution.

This analytical method has been the method of choice for determining formic acid in wood and in aqueous solutions. It has been applied to find the formic acid content in aqueous extracts of wood chips from the Vasa and it was used to determine the recovery of formic acid in the individual steps of the procedure for isolating formic acid from PEG-treated archaeological wood (section 4.3). It was also used to analyze the isotopic composition of formic acid in aqueous extracts of PEG-treated archaeological wood that had been spiked with labeled formic acid (section 4.2).

4.1.1 Experimental

The SPME needle was a Carboxen/PDMS Stableflex from Supelco (PDMS: polydimethylsiloxane, Carboxen: carbon based molecular sieve adsorbent) with an 85 μm coating. It was mounted in a Varian 8200Cx autosampler and the head-space-extraction time was 30 min. The GC used was a Varian 3400Cx with He 99.9995 % as carrier gas at a head pressure of 25 psi. The injector was an on-column injector with the following temperature program: initial 130 °C for 0.1 min, ramp to 250 °C at 300 °C min^{-1}, hold for 3 min. The column was a Restek Stabilwax-DA, L: 30 m, ID: 0.25 mm, coating: 0.25 mm. The oven temperature program was: initial 60 °C for 2.5 min, ramp to 230 °C at

30 °C min^{-1}, hold for 3 min. The transfer line to the MS was mounted with a No-Vent module at a temperature of 230 °C. The MS used was a Varian Saturn 2000 instrument with Silcosteel treated ion-trap electrodes (150 °C). The MS scanned from *m/z* 19 to 249, and averaged two scans every 0.5 s with SIS (Selective Ion Storage) to allow filtering of *m/z* 28 and 32. The pre-scan target TIC (Total Ion Count) for the ion trap was 5000. Chromatogram peaks were integrated at *m/z* 46+64+65 for deuterated acetic acid (CD_3COOD) and at *m/z* 29, 46 and 47 for formic acid. The MS instrument was autotuned. All calibration and analysis data were calculated as the ratio of peak areas for analyte/internal standard.

The procedure for analyzing formic acid in wood was described recently.[87] For the analysis of formic acid in aqueous solution, 100.0 μl of the sample solution was added to a 2 ml autosampler vial and mixed with 0.5000 ml of an aqueous 1 M sulphuric acid solution containing 5.000 μl/l d-acetic acid (CD_3COOD with 99.5% D, from Cambridge Isotope Laboratories). The headspace was analyzed by SPME GC-MS using the apparatus settings described. A series of standards was analyzed with every set of samples. The formic acid concentrations of these solutions were A: 0.246 g/l, B: 0.187 g/l, C: 0.123 g/l, D: 0.0615 g/l, E: 0 g/l. 100.0 μl of each of these solutions was put in a 2 ml autosampler vial and mixed with 0.500 ml aqueous 1M sulphuric acid solution containing 5.000 μl/l d-acetic acid.

4.1.2 Results and discussion

Figure 37A shows a representative chromatogram of standard solution C. The retention time is 5.65 minutes for the internal standard d-acetic acid and 5.85 minutes for formic acid. It is also seen that the chromatogram has small peaks at 5.30 and 5.95 minutes. They are background peaks found in the standard solution as well as in the samples. The analyte and the internal standard peaks were integrated over *m*/*z* 46, *m*/*z* 64 and *m*/*z* 65 for d-acetic acid and *m*/*z* 29, *m*/*z* 46 and *m*/*z* 47 for formic acid.

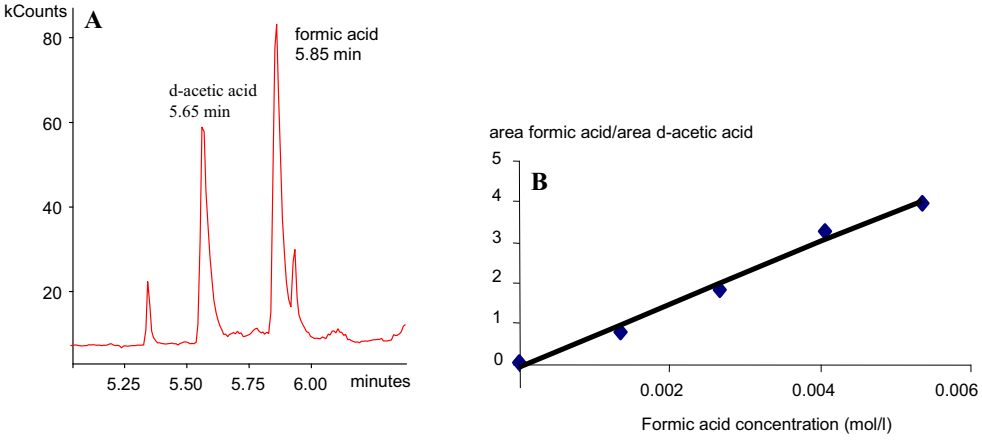


Figure 37. A: TIC of a standard solution (solution C). B: formic acid peak integrated over the sum of peaks at *m*/*z* 29, *m*/*z* 46 and *m*/*z* 47 divided by d-acetic acid peak area integrated over the sum of *m*/*z* 46, *m*/*z* 64 and *m*/*z* 65 plotted as a function of formic acid concentration. Recording is on the standard solutions A to E.

Figure 37B shows a representative calibration curve. The ratio of the named integral for formic acid and d-acetic acid is shown as a function of concentration. The data points are on a straight line and the linear regression is a good linear fit (r^2= 0.9896).

4.1.3 Conclusion

A method for analysing the formic acid content in aqueous solution has been tested successfully. The analysis measures formic acid in the headspace above a solution or suspension using an SPME needle and therefore it does not require the sample mixture to be injected directly into a GC or other equipment. This enables the measurement of formic acid in powdered solids, in samples of high-molecular matrices or other samples that would damage the equipment if injected directly into a GC.

4.2 Method for determining $H^{12}COO^-/D^{13}COO^-$ and $^{12/13}CO_2$ ratios

Isotope dilution experiments have been used to validate the methods for isolating formic acid from PEG-treated wood for radiocarbon analysis on the formic acid (section 4.3). A method was needed that could give the ratio of the non-labelled compound to the isotope-labelled compound, which, in this case, is $H^{12}COO^-/D^{13}COO^-$ and $^{12/13}CO_2$. Such a method for determining $H^{12}COO^-/D^{13}COO^-$ ratios was developed based on SPME GC-MS. A method for determining $^{12/13}CO_2$ ratios was developed for direct GC-MS analysis. There is no description in the literature of methods for determining $D^{13}COO^-/H^{12}COO^-$ ratios by SPME GC-MS. It is essential that the method can be carried out as an SPME-headspace analysis due to the sample matrix in question.

4.2.1 Experimental

The instrument used to determine the ratio of $D^{13}COOH$ to $H^{12}COOH$ was the same SPME GC-MS instrument described in section 4.1.1. The temperature programmes were also the same. For the analysis of $^{12/13}CO_2$, a different GC-MS instrument was used. This was a Varian 3400 gas chromatograph interfaced to a Saturn II ion trap mass spectrometer. The temperature of the transfer line (GC to MS) was 250 °C and that of the manifold of the mass spectrometer was 200 °C. The injector temperature was 100 °C and the column temperature was 50 °C isothermal. The column was a 25 m, 0.32 mm OD fused silica column coated with poraplot U (10 µm). It was operated with helium 99.9995 % as carrier gas with a 15 psi head-pressure on the column.

The procedure for analyzing ratio of $D^{13}COOH$ to $H^{12}COOH$ with SPME GC-MS was described in section 4.1.1. However, in this case, a series of formic acid standard solutions containing a mixture of non-labelled and isotopically-labelled formic acid were measured. The non-labelled formic acid was 98 % from J.T.BAKER, the isotope composition (natural composition) is; ^{12}C: 98.90 %, ^{13}C: 1.10 %, ^{1}H: 99.985 %, D: 0.015 %, ^{16}O: 99.762 %, ^{17}O: 0.038% and ^{18}O: 0.0200 %.[78] The labelled formic acid used was $D^{13}COONa$ from ISOTEC containing: min 99 atom % ^{13}C and min 98 atom % D. Non-labelled and labelled formic acid was mixed to give solutions where the fraction of labelled formic acid out of the total formic acid content was approximately 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% using Gilson pipettes. The total formic acid concentrations were almost identical for all the solutions (0.005345 M, 0.005353 M, 0.005362 M, 0.005371 M, 0.005379 M, 0.005388 M, 0.005396 M, 0.005405 M, 0.005413 M, 0.005422 M and 0.00543 M, respectively). The exact content of ^{12}C in relation to the total carbon in the formic acid solutions ($[^{12}C]/([^{12}C]+[^{13}C])$) were: 0.9890, 0.8897, 0.7907, 0.6920, 0.5937, 0.4956, 0.3979, 0.3004, 0.2033, 0.1065 and 0.0100 respectively. This number is referred to as the ^{12}C-fraction of the formic acid solutions. The ^{12}C-fractions listed here have been corrected for the 1.1 % natural ^{13}C content present in natural formic acid and for the 1% ^{12}C that is in the $D^{13}COONa$ isotope.

For analysing $^{12/13}CO_2$ ratios, the sample CO_2 is injected into the GC. The sample CO_2 is normally isolated in a vacuum-line after it has been produced by oxidation of formic acid (described in section 4.3.1.5). The sample CO_2 is allowed to expand into a ca. 125 ml gas pipette that has been evacuated first. The resulting pressure in the pipette should be approximately 10-20 mbar. The gas pipette is topped off with helium until atmospheric pressure is reached. A 500 µl Hamilton syringe is then pierced through the rubber membrane of the gas pipette and the plunger is pulled back and forth a few times to flush the syringe before it is filled with gas. Surplus gas is ejected out of the syringe until 30 µl is left, which is then injected on the GC. The retention time for CO_2 is 1.99 min. The peak is integrated with respect to the individual ions at *m*/*z* 44, 45 and 46 which is then processed as described in section 4.2.2.

4.2.2 Results and discussion

In Figure 38, three mass spectra are shown. They were recorded on formic acid with SPME GC-MS and the mass spectra are all taken from the formic acid peak in the chromatograms at retention time 5.95 min. The first spectrum (Figure 38A) is the mass spectrum of non-labelled formic acid; the content of labelled formic acid is 0 %. It is seen that the base peak is *m*/*z* 47 and then there are two more weak signals at *m*/*z* 48 and *m*/*z* 49.

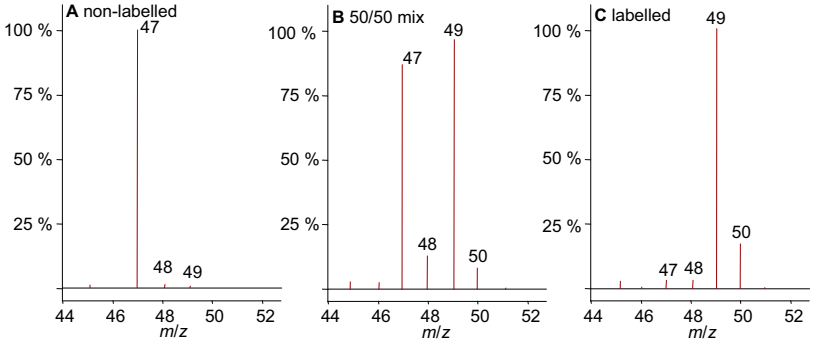


Figure 38. Examples of mass spectra recorded on $D^{13}C/H^{12}C$ - formic acid mixtures. Recordings of three different $D^{13}C$-fractions are shown: in A the $D^{12}C$-fraction is 0.99 corresponding to non-labelled formic acid, in B the $D^{12}C$-fraction is about 0.50 corresponding to a half and half mixture of non-labelled and labelled formic acid, in C the $D^{12}C$-fraction is 0.01 corresponding to a solution of the labelled formic acid (or $D^{13}COONa$) which is 99% pure in ^{13}C.

In Figure 38C, the spectrum of pure isotope-labelled formic acid is shown. The solution was prepared by dissolving pure $D^{13}COONa$ isotope (99%) in water. The base peak in this spectrum is *m*/*z* 49, but it also has weaker signals at *m*/*z* 47, *m*/*z* 48 and *m*/*z* 50. Figure 38B shows a mixture of the two isotopes where the labelled fraction is 50 %. It is seen that both *m*/*z* 47 and *m*/*z* 49 are intense, signals at *m*/*z* 48 and *m*/*z* 50 are also seen. Clearly the signal at *m*/*z* 47 relates to the non-labelled formic acid and the signal at *m*/*z* 49 is related to the labelled formic acid since these signals are base peaks in the spectra of the pure substances, however the peaks at *m*/*z* 48 and *m*/*z* 50 must be accounted for as well.

The ion at *m*/*z* 50 in the spectrum of pure labelled formic acid (Figure 38C) is particularly interesting because the only possible assignment is $[D_2H^{13}CO_2]^+$. $[D_3{}^{12}CO_2]^+$ also corresponds to an *m*/*z* 50, but this peak only appears in the spectra of labelled formic acid. Thus, assigning a ^{12}C ion to such an intense peak would not be correct. $[D_2H^{13}CO_2]^+$ contains two deuterium atoms, which demonstrates that exchange of protons takes place in the mass spectrometer since the additional deuterium atom in $[D_2H^{13}CO_2]^+$ can only come from another deuterated formic acid. Exchange of hydrogen atoms was also observed in the mass spectrometer by Pritchard et al.[93] It has also been shown that protonated formic acid exists in the gas-phase as a dihydroxo species.[94,95] Protonation and hydrogen atom exchange lead to a high number of possible assignments of ions in the present experiment, both D and H can bind to both ^{12}C and ^{13}C.

The possible reactions behind the exchange are listed in Figure 39A to H where EI (Electron Impact) produces radical cations of the formic acid in the sample, e.g. $[H^{12}COOH]^{+\bullet}$. $[H^{12}COOH]^{+\bullet}$

could abstract a deuterium atom from a $D^{13}COOH$ molecule in the mass spectrometer producing protonated formic acid $[H^{12}COOHD]^+$ and $[^{13}COOH]^\bullet$ (reaction A Figure 39). The latter species is not observed since it is uncharged, the former is seen at *m*/*z* 48. There is also the possibility where $[H^{12}COOH]^{+\bullet}$ abstracts the hydrogen atom from the oxygen atom of $D^{13}COOH$ (reaction B in Figure 39). This would lead to protonated formic acid at *m*/*z* 47.

A. $[H^{12}COOH]^{+\bullet}$ (46) + $D^{13}COOH \rightarrow H^{12}COOHD^+$ (48) + $[^{13}COOH]^\bullet$
B. $[H^{12}COOH]^{+\bullet}$ (46) + $D^{13}COOH \rightarrow H^{12}COOH_2^+$ (47) + $[D^{13}COO]^\bullet$
C. $[H^{12}COOH]^{+\bullet}$ (46) + $H^{12}COOH \rightarrow H^{12}COOH_2^+$ (47) + $[^{12}COOH]^\bullet$
D. $[H^{12}COOH]^{+\bullet}$ (46) + $H^{12}COOH \rightarrow H^{12}COOH_2^+$ (47) + $[H^{12}COO]^\bullet$
E. $[D^{13}COOH]^{+\bullet}$ (48) + $D^{13}COOH \rightarrow D^{13}COOHD^+$ (50) + $[^{13}COOH]^\bullet$
F. $[D^{13}COOH]^{+\bullet}$ (48) + $D^{13}COOH \rightarrow D^{13}COOH_2^+$ (49) + $[D^{13}COO]^\bullet$
G. $[D^{13}COOH]^{+\bullet}$ (48) + $H^{12}COOH \rightarrow D^{13}COOH_2^+$ (49) + $[^{12}COOH]^\bullet$
H. $[D^{13}COOH]^{+\bullet}$ (48) + $H^{12}COOH \rightarrow D^{13}COOH_2^+$ (49) + $[H^{12}COO]^\bullet$

Figure 39. Primary reactions in the mass spectrometer, *m*/*z* values are given in parenthesis.

It is seen that the reactions A to H in Figure 39 explain all the signals observed in the mass spectra. However, other reactions could lead to identical ions. For example, the protonated formic acid formed in the reactions A to H in Figure 39 could protonate other formic acid molecules. Another aspect is that the ion trap used here generates protonated molecules consistently. Thus a large number of secondary reactions are possible. It was demonstrated that these reactions take place in the mass spectrometer and not in the solutions. This was done by measuring mixtures of non-labelled and isotope-labelled formic acid and showing that the isotope ratio was constant over time (Appendix 5). This makes it possible to construct an empirical relationship between the ions determined in the MS and the fraction of a given carbon isotope.

In order to facilitate the quantification, an approximation was made in which all secondary reactions are ignored as well as the signals corresponding to the radical cations themselves. Furthermore, it is assumed that the reactions A to D in Figure 39 mostly relate to $[H^{12}COOH]^{+\bullet}$ and reactions E to H mostly relate to $[D^{13}COOH]^{+\bullet}$. Then we let the intensity of the masses *m*/*z* 47 and *m*/*z* 48 represent the concentration of ^{12}C formic acid ($[^{12}C]$), and the intensity of the masses *m*/*z* 49 and *m*/*z* 50 represent the concentration of ^{13}C formic acid ($[^{13}C]$). This way the ^{12}C-fraction can be expressed as:

$$^{12}C - fraction = \frac{[^{12}C]}{[^{12}C]+[^{13}C]} = \frac{A_{(47)} + A_{(48)}}{A_{(47)} + A_{(48)} + A_{(49)} + A_{(50)}}$$

where $[^{12}C]$ is the concentration of $H^{12}COOH$ in the solution and $A_{(m/z)}$ is the integral of the ion at *m*/*z* over the formic acid peak in the chromatogram. It is seen in Figure 40 that it is a good approximation. Here 11 solutions with different formic acid isotope ratios were measured three times on the SPME GC-MS and the ^{12}C-fraction calculated as described above. The experimental ^{12}C-fraction correlates well with the known ^{12}C-fraction. The linear regression of all the data points

in the three measurements of the series (red, blue and black points in Figure 40) has a total r^2 of 0.9958, which is acceptable.

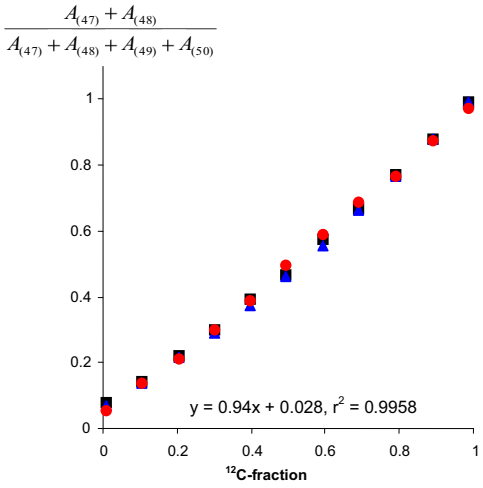


Figure 40. Calibration of the relative $H^{12}COOH$ and $D^{13}COOH$ contents in a series of solutions. They were measured on SPME GC-MS and the integrals of the individual ions were processed using the formula described ((47+48)/(47+48+49+50)). The series was measured three times (black, red and blue points in the curve).

It could be argued that the simplest and most straightforward relationship of ion intensities is:

$$^{12}C - fraction = \frac{A_{(47)}}{A_{(47)} + A_{(49)}}$$

This formula has been tested, it had a reasonable correlation of r^2=0.96, but for ^{12}C-fractions close to 0, the curve became non linear (data not shown).

A method for measuring isotope fractions in carbon dioxide $^{12/13}CO_2$ was also developed. A mass spectrum of a 50/50 mixture of $^{12/13}CO_2$ is shown in Figure 41.

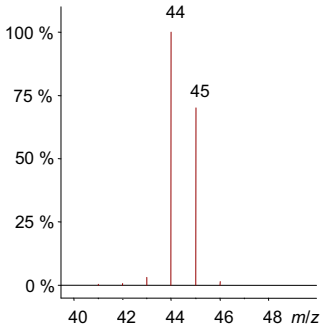


Figure 41. Mass spectrum of CO_2 containing a mixture of the ^{12}C and ^{13}C isotopes in approximately equal amounts.

In this mass spectrum three ions are observed namely *m*/*z* 44, *m*/*z* 45 and *m*/*z* 46 the latter signal is very weak. The peak at *m*/*z* 44 is assigned to $[^{12}CO_2]^{+\bullet}$ exclusively while the peak at *m*/*z* 45 is assigned to $[^{13}CO_2]^{+\bullet}$ possibly including a small amount of $[^{12}CO_2H]^+$, although protonation only occurs to a small extent in the MS used for these experiments. This is seen from the peak at *m*/*z* 46 which is assigned to $[^{13}CO_2H]^+$. This peak indicates the extent of protonation and it is very weak.

The assignment of ions is very simple in the case of carbon dioxide. Ignoring the (weak) protonation in the mass spectrometer, the $^{12/13}CO_2$-fraction is given by:

$$^{12}C - fraction = \frac{A_{(44)}}{A_{(44)} + A_{(45)}}$$

In this case no calibration has yet been made with standard mixtures of known $^{12/13}CO_2$ content. Instead SPME GC-MS measurements of (47+48)/(47+48+49+50) ion ratios were done on three formic acid isotope mixtures. The three solutions were then oxidized to carbon dioxide using the method described in section 4.3 and the ^{12}C-fractions of the CO_2 were measured as described above. The ^{12}C-fractions deduced in this way are listed in Table 3 with the number of measurements given in parentheses.

Table 3. ^{12}C-fractions

Sample	HCOOH ^{12}C-fraction (47+48)/(47+48+49+50)	CO_2 ^{12}C-fraction 44/(44+45)	Difference*100 (CO_2-HCOOH)
A	0.509(2)	0.557(5)	4.8
B	0.526(2)	0.562(5)	3.6
C	0.529(2)	0.571(5)	4.2
Average	0.522(6)[0.008]	0.563(15)[0.005]	4.1

Number of GC-MS measurements in parentheses, standard deviation in brackets.

It is seen that the difference between the two types of measurement is very small, 4.8 % is the largest difference between ^{12}C-fractions for the two. If the (47+48)/(47+48+49+50) number is corrected using the equation from the linear fit in Figure 40 the difference between the two types of measurement becomes even smaller. A small difference indicates that a possible contamination, isotopic fractionation or some systematic error, can only be minor. However, it does seem suspicious that the ^{12}C-fraction is higher in all the measurements of CO_2, than in any measurement of formic acid in Table 3. This increase in ^{12}C-fraction from formic acid to carbon dioxide could be an effect of the oxidation reaction, as discussed later.

4.2.3 Conclusions

An SPME GC-MS technique for measuring ^{12}C-fractions in solutions containing mixtures of $H^{12}COO^-$ and $D^{13}COO^-$ was tested successfully.

A GC-MS technique for measuring $^{12/13}CO_2$ ratios in gas was tested. It is not yet fully validated but it yields $^{12}CO_2$ -fractions that are close to the $H^{12}COOH$ fractions measured using the SPME GC-MS technique.

4.3 Method for isolating formic acid from wood

In order to analyse the radiocarbon signature of formic acid in PEG-treated archaeological wood, the formic acid must be isolated from the wood and purified before it can be subjected to AMS (Accelerator Mass Spectrometer) analysis. The formic acid concentration in PEG-treated archaeological wood is normally less than about 0.1%$^{w}/_{w}$.[87] The amount of sample needed for radiocarbon analysis by AMS is about 1 mg carbon. With a 100 % efficient purification, this would correspond to about 4 g of wood sample containing 0.1 % $^{w}/_{w}$ formic acid. Even though a long multistep isolation process has a recovery far below 100 % and many PEG-treated samples have less than 0.1 % formic acid in them, the sample size is realistic, at least for the Vasa.

Reactions between mercury(II)chloride and small organic molecules like formic acid have been described in the literature.[96-101] These include a selective oxidation of formic acid to CO_2 as described by Johnson[98] and later applied for atmospheric and environmental research.[96] The method takes advantage of the reaction of mercury(II)chloride with formic acid:

$$2HgCl_{2(aq)} + HCOOH_{(aq)} \rightarrow CO_{2(g)} + Hg_2Cl_{2(s)} + 2HCl_{(aq)}$$

It runs in the presence of silver perchlorate at pH<4 at 100 °C. The oxidation is described as selective for formic acid and this seems to be the case for the matrices in question. For example, Hornung[97] reacted a mixture of acetic acid and formic acid in this way without getting any products from the acetic acid but quantitative yield from the formic acid.

Using this reaction directly on an aqueous extract of PEG-treated archaeological wood chips, however, does not work. As shown in the section that follows, a three-step isolation procedure is necessary before the oxidation can function selectively. It involves a low-temperature vacuum distillation, followed by ion exchange and a second vacuum distillation. These procedures, along with the oxidation step, were validated in the following sections, for the matrix that is relevant to PEG-impregnated wooden shipwecks.

4.3.1 Experimental

4.3.1.1 Extraction

The procedure for extracting formic acid from PEG-treated wood starts with planing off wood shavings from a sample fixed in a vice lined with a plastic bag. The wood shavings are made by planing perpendicular to the grain of the wood so that they become very brittle. About 100 g of wood shavings are collected in a beaker. They are then put into a powerful 1½ l kitchen blender with approximately 300 ml ice made from HPLC-grade (High Pressure Liquid Chromatography) water in plastic bags. Wood and ice was added a little at the time and blended into a cold slurry. After blending, most of the ice was melted and the water could be decanted from the wood that is now powdered. The collected liquid is poured into centrifuge vials and centrifuged for 2 hours at 5400 rpm. The supernatant, which is black like coffee, is collected from the centrifuge tubes. At this stage about 150 ml liquid remains. The wood powder is poured into a Petri dish and left to dry.

4.3.1.2 Vacuum distillation

A drawing of the setup for vacuum distillation is shown in Figure 42. A 100 ml round-bottom flask containing the sample is frozen in the refrigerator and connected to trap number 1. A water bath with tap water allows gentle heating of the round-bottom flask. Trap 1 is connected to trap 2, both of which are submerged in liquid nitrogen (-196 °C).

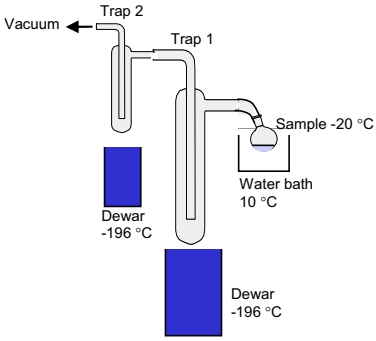


Figure 42. Setup for vacuum distillation. The frozen sample in a 100 ml round-bottomed flask is connected to two traps in series, trap 1 and trap 2. Vacuum is provided by an oil pump. The traps are submerged in dewars containing liquid nitrogen. The sample flask is heated with cold tap water.

The dimensions of the first trap are: diameter of outer tube: 5.8 cm, length of outer tube: 35 cm, clearance between bottom of the inner- and bottom of the outer tube: 6.5 cm. The dimensions of the second trap are: diameter of outer tube: 4 cm, length of outer tube: 25 cm, clearance between bottom of the inner- and bottom of the outer tube: 4 cm. Trap 2 is connected to a two stage Edwards high vacuum oil pump containing oil that has been changed within the last six months. The pump is equipped with an alumina foreline-trap with alumina that has been cleaned within the last six months (washed in acetone and baked out at 800-1000 °C for 3 hours).

Thirty ml of the aqueous extract of wood from the Vasa is mixed with 20 ml aqueous 2M H_3PO_4 (57.5 g of 85 % H_3PO_4 in a 250 ml flask topped off with HPLC-grade water) in a 100 ml round-bottomed flask. The flask is put in the freezer until it is frozen (over night). It is then mounted on the setup shown in Figure 42. The vacuum is turned on to the system and the 100 ml flask with the frozen sample is heated gently with cold tap water. Lukewarm water can be used with care as long as violent boiling of the liquid parts of the icy sample is avoided. The distillation is finished when the round-bottomed flask no longer gets cold, due to evaporation, when the water bath is removed (ca. 2-4 hours). At this point the vacuum must be stopped. The aqueous formic acid in trap 1 is thawed and collected. At this stage the formic acid content can be measured by SPME GC-MS in which case the collected sample is transferred to a 100.00 ml volumetric flask, which is then topped off with water and measured. If knowing the formic acid content is not required, the collected solution is just transferred to the next step, which is ion exchange. Solutions with formic acid contents between 0 and 0.15 g/l have been processed successfully.

Only 30 ml of extract can be distilled at one time. Therefore a low formic acid content in the extract may require that several distillations must be made in order to isolate enough formic acid.

4.3.1.3 Ion exchange

The equipment used for ion exchange consists of a 50 ml reservoir mounted on top of the ion exchange column. The ion exchange column is a L45 mm, Ø12 mm column packed with PSA (Primary Secondary Amine) bonded silica from Supelco. The column is fitted on a vacuum chamber that allows for collection of the liquid eluting from the column.

The columns were packed with PSA-bonded silica by mixing this powder with HPLC-grade water to give a thick flowing suspension which was carefully poured into the column tube (with a filter at the bottom) and left to settle for a few minutes. Vacuum was applied and more suspension was added until the tube was sufficiently full to allow the top filter and cap to come on. The column is conditioned by letting 15 ml HPLC-grade water run through. The vacuum is adjusted to give a flow of approximately one drop per second. Then 10 ml of 2M aqueous NH_3 solution and finally HPLC-grade water are run through until neutral pH is obtained in the liquid eluting from the column (after approximately 150 ml water).

The sample solution containing the formic acid can then be applied followed by 10 ml HPLC-grade water followed by a 50 ml portion of HPLC-grade water. The container in the vacuum chamber is then emptied and 10 ml of 2M NH_3 solution is passed through the column and then 10 ml HPLC-grade water followed by 20 ml HPLC-grade water. The container in the vacuum chamber now contains the sample formic acid. If the formic acid content must be analysed, then the content is transferred to a 50.00 ml volumetric flask that is topped of with HPLC-grade water. The formic acid content can then be measured using the SPME GC-MS technique. Otherwise the liquid is transferred to the next step in the purification which is the second vacuum distillation.

4.3.1.4 Second vacuum distillation

The second vacuum distillation is performed like the first one. Only 30 ml of the solution collected after ion exchange can be processed at one time because of the dimensions and capacity of the trap. For this reason it is necessary to distil twice in order to process the entire volume of sample.

4.3.1.5 Oxidation

For oxidation of formic acid, a setup is used where the reaction takes place in a round-bottom flask that is connected to a vacuum line that is connected to an oil vacuum pump. This is illustrated Figure 43. A 3-neck round-bottom flask is charged with a magnetic stir bar and the formic acid fraction collected in the second vacuum-distillation. The following substances are then added in named order: 25 μl 37 % HCl (pH of the solution is 2), 0.5 g $AgClO_4 \cdot H_2O$ (99 %) and 1.0 g $HgCl_2$ (99.5 %). The flask is closed and connected to the rest of the setup and a thermostat-controlled oil-bath is placed under the flask on a heating plate with magnetic stirring. Helium (99.9995 %) is constantly bubbled through the contents of the reaction flask constantly and it leaves the flask through a reflux condenser with a temperature between 1 and 5 °C, which keeps water from escaping from the flask. This temperature is maintained by a thermostatic cooler that pumps an antifreeze liquid through the reflux condenser. The gas then passes through the U-tube and into V_B, which is closed to the rest of the vacuum-line (valves 2, 3 and 4 open, valve 1 and 5 closed Figure 43) It leaves the setup through valve 4. The flask V_A is not on the vacuum-line while the reaction is taking place. The helium flow is adjusted to approximately 80 ml/min., measured at the exit of the vacuum line through valve 4 (atmospheric pressure). Helium is allowed to flow through the system for 5-10 minutes in order to flush the system before liquid nitrogen is poured into the dewar under the U-tube and the temperature is raised to 100 ºC in the oil bath. The reaction then proceeds for three hours after the temperature has reached 100 ºC.

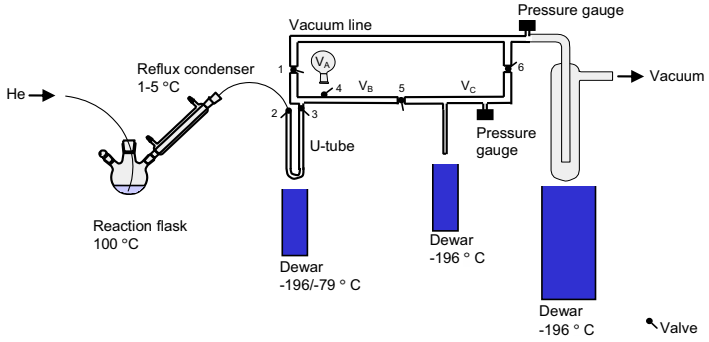


Figure 43. Drawing of the setup used in the oxidation of formic acid to carbon dioxide. Carbon dioxide is produced in the reaction flask. A flow of helium takes the carbon dioxide to the U-tube (-196 °C) where it condenses. After the reaction has finished the carbon dioxide in the U-tube is distilled from the U-tube (now -79 °C) into the test tube (-196 °C) in V_C. Thawing the carbon dioxide allows determining the yield by reading the pressure in the known volume of V_C. The carbon dioxide is transferred to a glass ampoule (not shown) on V_B at –196 °C, which is sealed and melted off using a ring burner. The Valves are numbered from 1 to 6.

The CO_2 that is produced in the reaction passes through the reflux condenser and condenses in the U-tube (photographed in Figure 44). The temperature in the U-tube is -196 °C, cold enough to freeze the produced CO_2 (condenses at –79 °C), but not the excess helium (bp –272 °C). When the reaction is finished, the U-tube is closed to the condenser and reaction flask (valve 2 closed), the helium is turned off, and valves 1, 4 and 6 are closed and valve 5 is opened. Then vacuum is applied (valve 6 is opened) to remove helium (the frozen CO_2 stays in the U-tube). Valve 6 is closed when the pressure indicates that all helium is removed. Then the dewar containing liquid nitrogen around the U-tube is replaced by a dewar containing a mixture of dry ice and ethanol (-79 °C). In this way, the trapped CO_2 distils from the U-tube into V_B and V_C which have both been evacuated. The pressure increases as the CO_2 evaporates. When the pressure stops increasing, the distillation has finished. Then liquid nitrogen is placed under the test tube in V_C and the CO_2 condenses here while the pressure drops. When the pressure gauge reads zero, all the CO_2 is trapped in the test tube. Then valve 5 is closed and the test tube is thawed. The pressure in V_C increases and, since the volume of V_C is known, the yield of CO_2 can be calculated. The U-tube is taken off the vacuum line and replaced by a glass ampoule (L app. 22 cm when sealed, OD 8mm, ID 5.2 mm) using a “Cajon union” o-ring system on valve 3. V_B, now including the ampoule, is evacuated (by opening valve 1 and closing it again when the pressure gauge reads zero mbar). Valve 5 is then opened, the ampoule is cooled with liquid nitrogen and the CO_2 is trapped in the ampoule, which is then sealed and melted off using a gas/air ring burner. The sealed glass ampoule is sent off for analysis at the AMS facility in Kiel, Germany.

The volume of V_C, which includes the volume of the test tube, is normally around 117 cm^3. This volume can change a little if the test tube has been taken off or new grease has been applied to it, but it can always be calibrated using a flask with a known volume. In this case V_A is a flask with a known volume, V_A=121.46 ml, determined by weighing it with and without distilled water at room temperature. If a certain pressure (P_1) of air is established in V_A (say 100 mbar) then this represents an amount of gas V_AP. If V_B and V_C are evacuated and the gas in V_A allowed to expand into the total volume of V_B and V_C (by opening valve 4), then the new pressure (P_2) can be read on

the pressure gauge in V_C. Assuming that V_B and V_C were completely empty after evacuation, the new pressure (P_2) allows the calculation of the total volume V_B+V_C since $P_1V_A=P_2(V_A+V_B+V_C)$. Using the same principle for V_C expanding into V_B allows the calculation of V_C using the total volume of V_B+V_C and the relation $P_1V_C=P_2(V_C+V_B)$.

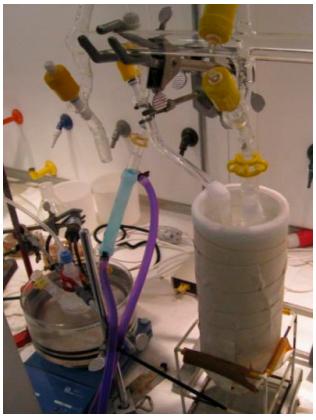


Figure 44. Photograph taken during oxidation of sample formic acid. The U-tube in the foreground is submerged in liquid nitrogen in a dewar to trap carbon dioxide. The reaction flask which is seen in the background with the reflux condenser (blue tubes).

4.3.1.6 Sample pre-treatment and AMS

Radiocarbon measurement by AMS and the AMS pre-treatments described in this section were performed by the staff of Prof. Dr. P.M. Grootes, at Leibniz Labor für Altersbestimmung und Isotopenforschung, Christian-Albrechts-Universität, Kiel, Germany.

The CO_2 from the ampoule was graphitized with H_2 and Fe as a catalyst at 600°C. The iron-graphite mixture was pressed into an aluminium target holder and measured on the AMS.

The ^{14}C concentration of the sample was measured by comparing the simultaneously collected ^{14}C, ^{13}C, and ^{12}C beams of each sample with those of Oxalic Acid standard background material. Conventional ^{14}C ages were calculated according to Stuiver and Polach[92] with a $\delta^{13}C$ correction for isotopic fractionation based on the $^{13}C/^{12}C$ ratio measured by the AMS-system simultaneously with the $^{14}C/^{12}C$ ratio (note: This $\delta^{13}C$ includes the effects of fractionation during graphitization and in the AMS-system and, therefore, cannot be compared with $\delta^{13}C$ values obtained via mass spectrometer on CO_2). The standard deviation (σ) contains both the counting statistics of the ^{14}C measurement and the variability of the interval results that, together, make up one measurement. The larger of the two is adopted as the measuring uncertainty. The uncertainty connected with the subtraction of the “blank” is added. The quoted 1σ uncertainty is the best estimate of the full measurement and is not just based on counting statistics. “Calibrated” or calendar ages were calculated using “CALIB rev 5.01” (Data set: IntCal04[90]).

It has been necessary to measure the ^{14}C content of pure formic acid, pure PEG and components from wood (explained in section 4.5). Such samples were combusted to CO_2 in a closed quartz tube together with CuO and silver wool at 900 °C. The sample CO_2 was reduced with H_2 over about 2 mg of Fe powder as catalyst, and the resulting carbon/iron mixture was pressed into a pellet in the target holder.

It has been necessary to measure the ^{14}C content of wood components in PEG-treated archaeological wood (explained in section 4.5). This involves washing away unwanted components such as PEG, from the wood, prior to measurement. First the samples were checked and mechanically cleaned under the microscope. To remove organic contaminants such as PEG, the samples were first extracted 3 times with hot water (85°C) and then subjected to a soxhlet-type serial extraction to remove additional nonpolar to polar organic contaminants. In sequence, they were extracted three times each with boiling tetrahydrofuran (THF), chloroform, petroleum-ether, acetone, and methanol and then rinsed with demineralized water (this step corresponds to the samples called SPAN1 in Table 7). Then the sample material was extracted with hot water (3 times at 100 °C in a soxhlet) and subsequently extracted with 1 % HCl, 1 % NaOH at 60 °C and again 1 % HCl (this step corresponds to the samples called SPAN2 in Table 7). Then another 5 extractions with hot water at 85 °C were performed (this step corresponds to the samples called SPAN3 in Table 7). The sample material was dried and flame sealed in a closed quarz tube for combustion as described above. The ^{14}C content was almost unchanged after the last aqueous extraction but not completely (Table 7).

4.3.2 Results and discussion

The aqueous extract containing formic acid from the PEG-treated wood sample is first vacuum distilled. This step is referred to as VD1. The product is thawed and purified on a weak anion exchange resin; this step is referred to as IE. This solution is then vacuum distilled a second time to remove ammonia used in the ion exchange; this step is referred to as VD2. The sample formic acid solution thus collected is oxidised to carbon dioxide in the reaction with mercury(II)chloride described; this step is referred to as OX. The carbon dioxide is isolated in a glass ampoule that is sent off to the AMS laboratory in Kiel.

A test was set up to evaluate this series of procedures. A sample of wood from the Vasa was extracted as described (section 4.3.1.1). The concentration of formic acid in this extract was determined using the SPME GC-MS procedure as described (section 4.1.1). An amount of $D^{13}COONa$, approximately equal to the amount of formic acid determined in the extract, was added. The resulting solution is referred to as SPIKED. This gave a theoretical ^{12}C-fraction of 0.45 and a total formic acid concentration of 0.00921 M including both non-labelled and isotope-labelled formic acid. The sample was divided into three samples and each of these were subjected to the entire series of isolation steps from VD1 to OX.

^{12}C-fractions were measured using the SPME GC-MS technique described in section 4.2.1. after VD1, IE and VD2. The ^{12}C-fraction was calculated using the (47+48)/(47+48+49+50) relation described, but it was corrected using the equation from the linear regression shown in Figure 40. The total formic acid content was determined from these chromatograms and the recovery was calculated. The ^{12}C-fraction after the oxidation (OX) was determined using the *m*/*z* 44/(44+45) relation without any correction, as described in 4.2.1. The recovery after OX is based on the pressure of CO_2 measured in the vacuum-line. The results of this test are illustrated in Figure 45.

The average of the recoveries of the three solutions, after each of the isolation steps is shown in the top graph of Figure 45. It is seen that the three unprocessed extracts with isotope, called

SPIKED in Figure 45, have an average recovery of 112 % (standard deviation σ=12 over the three measurements, red column in Figure 45). This number also contains the inaccuracy that is connected to the determination of the formic acid content in SPIKED before the $D^{13}COONa$ was added. After the first vacuum distillation, the recoveries averaged 74 % (σ=3.1), 105 % (σ=5.6) after the ion exchange, 99 % (σ=10) after the second vacuum distillation and 90 % (σ=6.2) after the oxidation. These recoveries are all quite high and the standard deviations within the three measurements no higher than 12 %. Thus the four steps in the isolation procedure are reasonably effective. The total recovery over all the steps from the theoretical SPIKED (100 %) to OX is 69 %.

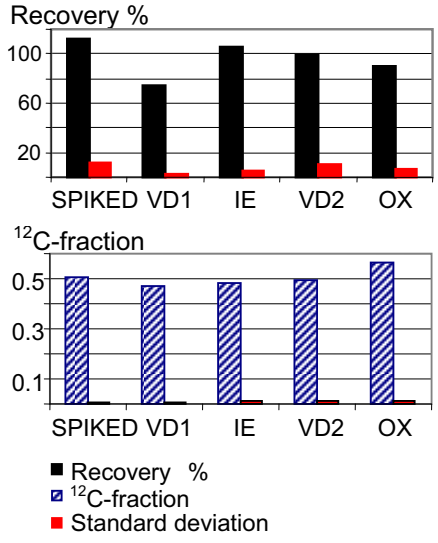


Figure 45. Top: Black columns represent the recovery (average over three samples). The recovery is shown for the four steps in the purification: first vacuum distillation (VD1), ion exchange (IE), second vacuum distillation (VD2) and oxidation to CO_2 (OX), SPIKED is the name of the initial formic acid solutions (where $D^{13}COONa$ has been added). Bottom: blue columns (hatched) represent ^{12}C-fractions in the purification steps (average over three samples). The standard deviation is shown as red columns.

The necessity for the isolation steps became clear when a sample of aqueous extract from the Vasa, which also contained $D^{13}COONa$, was oxidised directly. This resulted in an increase of the ^{12}C-fraction from 0.45 in the initial solution to 0.62-0.64 in the carbon dioxide that resulted. Thus the sample matrix contains components that can be oxidised to carbon dioxide. It is shown here how to remove them in a series of procedures (from SPIKED to VD2). In the bottom of Figure 45 the averages of the triplicate measurements of the ^{12}C-fractions are shown for the steps in the isolation. It is seen that the ^{12}C-fractions stays between 0.56 (OX) and 0.47 (VD1), and that the standard deviations are no higher than σ=0.012. The only step where there may be a slight change in the ^{12}C-fraction is the oxidation (OX). There is an increase in the ^{12}C-fraction of 0.04. This is a minor change only but it is an increase in the ^{12}C content the natural carbon isotope, not in ^{13}C. The ^{12}C increase could be due to a contamination of the reaction mixture in the oxidation, with natural carbon. If for example carbon dioxide from the air had dissolved in the aqueous sample solution (as bicarbonate, by accident) and subjected to the oxidation, it would increase the ^{12}C-

fractions. This seems unlikely since the solution was purged with helium before the reaction was started and the reaction mixture was acidic which should drive out carbon dioxide. Another source of the ^{12}C increase could be the sample matrix. All carbon in the Vasa is natural with respect to $^{12/13}C$, thus if a component from the Vasa other than formic acid is converted to CO_2 in the oxidation step then it would increase the ^{12}C-fraction. The third explanation could be isotopic fractionation. Physical or chemical reactions that are not allowed to run to completion may induce isotopic fractionation. In the oxidation of formic acid, breaking of D-^{13}C bonds would take place at a lower rate than the breaking of H-^{12}C bonds. This would result in an increase in the ^{12}C-fraction. However the isotope effect should be small in the present case. There could also be another explanation for the ^{12}C increase. The ^{12}C-fractions of the carbon dioxide in OX and the formic acid in VD2 were determined using different GC-MS equipment and techniques, which could also be a source of the discrepancy.

It cannot be rejected that the increase in ^{12}C-fraction of 0.04 over the oxidation step is an indication that a small amount of foreign carbon, maybe from the matrix, enters the sample here. If the increase in ^{12}C-fraction is a reality, then the contamination is small. However, the ^{12}C-fractions are generally stable through the 5 steps of isolation as seen in Figure 45 bottom.

4.3.3 Conclusions

A four step procedure for isolating formic acid carbon as CO_2 was evaluated. It involves vacuum distillation, ion exchange, a second vacuum distillation and a reaction with mercury(II)chloride. The recovery is reasonable in each of the steps. The total recovery is 69 % over all steps.

Processing a mixture of D^{13}COO$^-$ and H^{12}COO$^-$ yielded almost constant ^{12}C-fractions over all the steps in the isolation. This indicates that the procedure is selective for formic acid and that almost no foreign carbon contaminates the sample in any of the steps. The largest possible contamination would correspond an increase in the ^{12}C-fraction of 0.04 over the oxidation.

4.4 Formic acid content in PEG-treated wooden shipwrecks

If formic acid is the primary product of PEG degradation, as suggested in Chapter 3, then detecting increased amounts of formic acid in PEG-treated wooden objects would indicate that PEG degradation has taken place. Clearly some formic acid could evaporate from the object resulting in predictions of PEG degradation that are too low. Another factor could be release of formic acid by the wood itself; elevated formic acid contents would lead to predictions of PEG degradation that are too high.

Information about formic acid content in archaeological wood is very scarce in the literature. However, fresh wood has been studied in more detail. As explained in the introduction there are indications that formic acid is a naturally occurring component of fresh wood although perhaps not under museum conditions. In spite of the uncertainties regarding the possible sources of formic acid in PEG-treated wood, this chapter deals with measurement of formic acid. Formic acid contents in different PEG-treated wooden shipwrecks, from an article[87] and from measurements in the present work, are discussed in this section.

4.4.1 Experimental

The procedures used in this chapter were described in section 4.1.1 and in Glastrup *et al.*[87]

4.4.2 Results and discussion

The formic acid content in PEG-treated archaeological wood samples from different sources have been measured by SPME GC-MS. Some of these measurements are shown in Figure 46. This Figure was modified after Glastrup *et al.*[87] and it shows the formic acid contents of a number of samples from four different ships. The Vasa, the Oberländer boat, the Skuldelev Viking ships and the Bremen cog were sampled. They contained an average of 0.031 % formic acid by weight (σ=0.015), 0.012 % (σ=0.0015), 0.030 % (σ=0.014) for the PEG-treated planks of the Skuldelev ships and 0.063 % (σ=0.029) for the PEG-treated planks of the Bremen cog, respectively.

There is a large variation between the measurements of formic acid in different parts of the ships, for example the standard deviation (σ) of the measurements of the Vasa samples was 0.015 corresponding to 47 % of the mean formic acid concentration. That is a large deviation, but it should be remembered that the samples are from different parts of the ships and there is nothing to indicate that they should contain equal amounts of formic acid. For example, Table 4 lists formic acid contents measured both under the wood surface and in the surface of the same samples from the Vasa. These numbers are not identical even though they were measured in the same piece of wood. It is noticed that the formic acid content is higher in the surface than it is under the wood surface. This is interesting because there is usually more PEG in this part of the wood, and PEG may be the source of the formic acid. However there are not enough measurements here to draw definitive conclusions about the distribution of formic acid in the wood as a function of sampling depth below the wood surface.

The variation of the formic acid contents among the different ships analysed is not much larger than the variation between samples in one ship (such as the Bremen cog). They contain between 0.012 % and 0.063 % formic acid on average. The three samples from the Bremen cog that had not been treated with PEG contained an average of 0.032 % formic acid (σ=0.019) and the two samples from the Skuldelev ships that had received no PEG-treatment contained 0.0065 % (σ=0.0005).

These formic acid contents are low but still within the range of the samples containing PEG, at least for the Bremen cog; for the Skuldelev ships they are a little lower (black bars in Figure 46). This indicates that formic acid is present in archaeological wood naturally, but it is not impossible that PEG degradation has contributed to the amount of formic acid. In other words, the formic acid content in PEG-treated wood is equal to or slightly elevated compared to archaeological wood that has not received PEG-treatment.

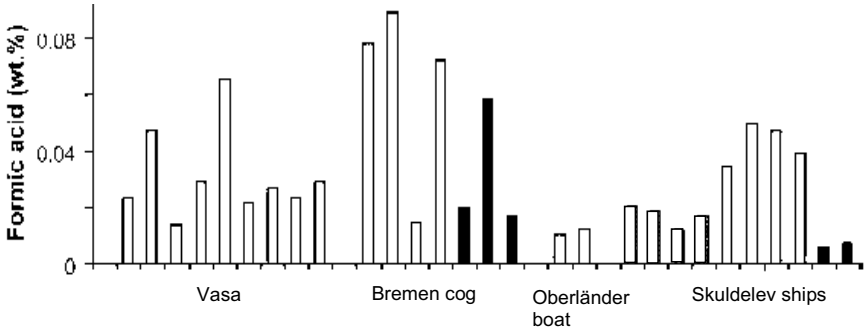


Figure 46. Formic acid contents in different samples from wooden shipwrecks. All samples have received PEG-treatment except for those indicated by Black bars which represent waterlogged archaeological wood that has not received any PEG-treatment. Figure after Glastrup *et al*.[87]

In general, the absolute formic acid contents are in agreement with the values calculated from the aqueous extracts, as shown in Table 4. Here, three samples from the Vasa were extracted with water as described in section 4.3.1.1. The extraction procedure used here is the same procedure used for isolating formic acid for radiocarbon measurement. The formic acid content of the aqueous extract was measured and the formic acid concentration of the extracted wood was calculated, assuming that all formic acid had been extracted. This method gave concentrations between 0.02 % and 0.06 % formic acid by weight in the wood, which is similar to the values shown in Figure 46.

Table 4. Formic acid concentrations in samples from the Vasa

Sample	Formic acid content in wood $\%^{w}/_{w}$ (measured directry on the wood by SPME GC-MS)	Formic acid content in wood $\%^{w}/_{w}$, (measured indirectly via formic acid concentration in aqueous extract)
Vasa_A	Core: 0.090, Surface: 0.14	**0.05 $\%^{w}/_{w}$**
Vasa_B	Core: 0.068, Surface: 0.084	**0.02 $\%^{w}/_{w}$**
Vasa_C		**0.06 $\%^{w}/_{w}$**

However, they are lower than the values found using the direct SPME GC-MS method both on the surface and in the core of the two samples measured in Table 4. This suggests that not all of the formic acid is extracted from the wood by the extraction procedure described in section 4.3.1.1.

The average content of formic acid in the Skuldelev Viking ships was 0.030 % and it was 0.031 % for the Vasa. Since formic acid was a product of the accelerated PEG degradation experiments

described in Chapter 3, it is relevant to ask how much PEG degradation this amount of formic acid corresponds to. As mentioned already, the fact that formic acid is found in archaeological wood that has received no PEG-treatment demonstrates that not all of the formic acid can be a product of PEG. It is also clear that a certain amount of formic acid must have evaporated from the wooden objects over time. If this is ignored and it is assumed that all of the formic acid that is in the wood corresponds to PEG degradation, then the life span of the PEG-impregnation of the Vasa and the Skuldelev ships can be estimated, as shown in Table 5.

Table 5. PEG lifespan based on formic acid concentrations

	Average HCOOH content from ref.[87]	Mole EG monomer degraded per kg wood sample*	PEG-impregnation	PEG content Estimated from ref.[102]	Total moles EG monomer per kg wood sample	Percent EG monomers degraded	Rate of degradation %/year**	Life span of PEG-impregnation
Skuldelev	0.030 % $^{w}/_{w}$	3.26 mmol/kg	PEG 4000 $\overline{DP}$=90.5	25 % $^{w}/_{w}$	5.66 mol/kg	0.058 %	0.0015 %/year	69000 years
Vasa	0.031 % $^{w}/_{w}$	3.37 mmol/kg	PEG 600:1500 1:1 $\overline{DP}$=13.2 and $\overline{DP}$=33.7	25 % $^{w}/_{w}$	5.56 mol/kg	0.061 %	0.0013 %/year	76000 years

*Assuming that two HCOOH are produced for every EG monomeric unit (-O-CH_2CH_2-) degraded in PEG.
**Assuming that degradation has taken place between the onset of conservation and 2008, 46 years for the Vasa and an estimated 40 years for the Skuldelev Viking ships.

The formic acid concentration in the Skuldelev Viking ships was 0.030 % on average. If the stoichiometry from Scheme 2 is adopted here: assuming that all formaldehyde produced in the degradation is oxidised to formic acid, then two formic acid molecules are produced per ethylene glycol (EG) repeating unit. This implies that 3.26 mmole of the EG monomeric units must have degraded in a kg of this sample material. The Skuldelev Viking ships have been treated with PEG 4000 (average Degree of Polymerisation, $\overline{DP}$=90.5) only and the concentration of PEG in a sample varies between 80 % on the surface and 10 % deeper inside the wood.[102] On this basis, it is estimated that 25 % of a wood sample consists of PEG. This means that one kilo of sample contains 5.66 moles of EG monomeric units. Using the formic acid content, it is seen that 0.058 % of the EG monomeric units present in the Skuldelev ships initially must have degraded. This leads to a yearly loss of 0.0015 % of the EG that was in the wood initially and that it will take 69000 years before all the EG monomeric units in the Skuldelev ships have been transformed into formic acid (see Table 5).

A similar estimate is shown for the Vasa in Table 5. Assuming that this wood contains PEG 600 ($\overline{DP}$=13.2) and PEG 1500 ($\overline{DP}$=33.7) in a 1:1 ratio (ignoring the PEG 4000 that is also present in a limited amount), this leads to an estimated PEG lifetime of 76000 years. The PEG contents of the Bremen cog and the Oberländer boat are not known, but they are likely to be similar to the Vasa and the Skuldelev ships and since the formic acid contents are also similar, the PEG life expectancies are probably also similar.

70000 years is a very long time. If the formic acid that is in the wood naturally is taken into consideration then an even smaller fraction of the formic acid can be attributed to PEG degradation and the number of years it takes to degrade PEG in the ships becomes even longer. The interpretation of these calculations would be that no significant PEG degradation takes place.

In the calculated example, PEG degradation was assumed to have taken place during the time that had elapsed since the onset of conservation (46 years for the Vasa). There could have been periods during this time span where degradation would have been more likely to take place. For example, in the case of the Vasa, the PEG was applied as a mist of aqueous PEG solution, so there must have been plenty of oxygen available for PEG degradation. It has also been shown in this study and in others that PEG degrades when heated. This means that the use of hot air blowers to melt off surplus PEG from the Vasa and the use of heat in the impregnation tanks for the Skuldelev ships could have been the cause of the degradation that corresponds to the formic acid found in the wood.

One interpretation of the formic acid content has not yet been considered, and that is continuous formation and evaporation of formic acid. The vapor pressure of formic acid is almost the same as that of water.[78] Thus formic acid is expected to evaporate from wood at room temperature, at least to some extent. If the formic acid concentrations in the static interpretation discussed above are really steady-state concentrations in a dynamic system where formic acid is produced and evaporates continuously, then the conclusion would be that PEG degradation is taking place and the lifespan of the PEG-impregnations in the ships is lower than 70000 years.

4.4.3 Conclusions

The formic acid content was measured in PEG-treated wooden shipwrecks. It was 0.031 % by weight in samples from the Vasa, 0.012 % in the Oberländer boat, 0.030 % in the Skuldelev Viking ships and 0.063 % in the Bremen cog. These formic acid contents were equal to or slightly elevated compared to archaeological wood that had not received PEG-treatment (measured for the Bremen cog and the Skuldelev ships).

If this formic acid content is translated into PEG degradation since the onset of conservation, then it would take approximately 70000 years to degrade the PEG that is estimated to be in the Vasa or the Skuldelev ships. If the formic acid evaporates continuously, then the measured formic acid contents are steady-state concentrations and the PEG-impregnations would be degrading faster.

4.5 Radiocarbon analysis of formic acid in the Vasa

The experiments presented in this section use the radiocarbon technique to try to find out if formic acid in the Vasa is a product of PEG degradation or of the wood itself. PEG is normally a petrochemical which is ^{14}C-depleted. At least one of the major PEG manufacturers, uses ethylene oxide in the production which itself is a typical petrochemical. This was also discussed in the introduction of Chapter 3 (Figure 13).[23] The Vasa sank in 1628 just after it had been built. The ^{14}C content of the wood should correspond to the years up to 1628 (96 pmC, percent modern Carbon). The ^{14}C content of formic acid reflects its origin: if formic acid in the Vasa is a product of PEG degradation then it is ^{14}C-depleted, while if it is a product of the Vasa wood it has a pmC of 96 like the wood. If both are sources of formic acid then the ^{14}C content will be intermediate.

It is the aim here to compare the ^{14}C content of formic acid isolated from the Vasa with the ^{14}C contents of wood and PEG from the Vasa. For the purpose of this comparison, the Percent Petrochemical (PP) is introduced. This number shows how much a sample resembles PEG which has a PP of 100 since it is completely petrochemical and, in the case of the Vasa, 0 PP means that the sample resembles the Vasa timbers completely (pmC=96, RCA=322 BP, CA=1628). This is illustrated in Figure 47 where four different scales are compared.

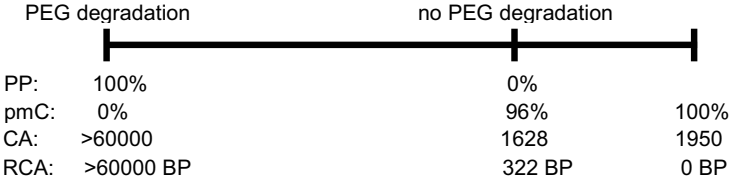


Figure 47. Comparison of four different scales: PP (percent petrochemical), pmC (percent modern Carbon), CA (calendar age) and RCA (radiocarbon age), to represent ^{14}C content of formic acid from the Vasa. Formic acid with 100 PP indicates PEG degradation, 0 PP indicates that no PEG degradation has taken place. RCA and CA corresponding to 0 pmC is set to 60000 years in this illustration because this is the ultimate limit for an AMS measurement.

PP (percent petrochemical), pmC (percent modern Carbon), CA (calendar age) and RCA (radiocarbon age), are shown in relation to formic acid in the Vasa. Formic acid with 100 PP indicates that only PEG has degraded in the sample, while 0 PP indicates that no PEG degradation has taken place and that the source of the formic acid is the wood components rather than PEG. The RCA and CA corresponding to 100 PP is set to more than 60000 years because this is the absolute limit of the AMS technique when dating old organic material. In the present chapter, formic acid is isolated from a Vasa sample and the PP is determined.

4.5.1 Experimental

The results presented here were obtained by following the procedures described in section 4.3.1.

4.5.2 Results and discussion

It has been assumed until now that PEG is a petrochemical because it is normally produced from petrochemicals. There has been some uncertainty about this assumption some of the manufacturers of the PEG used to impregnate the Vasa could have used substances from wood in the production. Clearly this would make it hard, or maybe impossible, to use the ^{14}C content of formic acid to establish its origin. For that reason three samples of PEG left over from the impregnation of the Vasa were analysed by AMS. The results are shown in Table 6. Here radiocarbon measurements are shown for a batch of PEG 1500 and PEG 4000 supplied by Mo & Domsjö and a batch of PEG 4000 supplied by Berol AB. It is seen that they are all completely petrochemical. The pmC values are between 0.06 and 0.17 which correspond to an almost complete ^{14}C-depletion. The average of these three measurements was used to set the PP scale for 100; it seemed sensible to use real measurements for this rather than setting 100 PP= 0 pmC.

Table 6. Carbon isotope analysis of PEG

	Sample Description	Corrected pmC†	Conventional Age	$\delta^{13}C$(‰)‡	PP*
PEG1	PEG 1500 from Mo & Domsjö AB	0.06±0.04	> 52670 BP	-26.21±0.13	>100.01
PEG2	PEG 4000 from Berol AB	0.13±0.04	53160 (+3050 / -2210) BP	-26.81±0.25	100.02
PEG3	PEG 4000 from Mo & Domsjö AB	0.17±0.05	51120 (+2600 / -1960) BP	-26.00±0.14	99.98

†"Corrected pmC" indicates the percent of modern (1950) carbon, corrected for fractionation using the ^{13}C measurement.
‡ $\delta^{13}C$ includes fractionation due to sample pre-treatment and to AMS, as described in section 4.3.1.6.
*The average of these three measurements was used to set the PP scale to 100%.

The wood components and formic acid were analysed by AMS in two samples from the Vasa called samples A and B. The wood chips left after formic acid had been extracted, were washed (section 4.3.1.6) until the ^{14}C content was stable. This is shown in Table 7, where the wood chips from sample A are called A_SPAN. It is seen that the corrected pmC ends up at 93.56 for sample A_SPAN3 and 96.18 for sample B_SPAN3 after the three extraction series (SPAN1 to 3). These values are close to the theoretical values for the Vasa. The conventional ages that correspond to these pmC´s are 535 BP and 315 BP respectively. The Vasa sank in 1628, corresponding to 322 BP. If it is assumed that the trees had a certain age before they were used for building the Vasa, then these numbers seem realistic. It should be mentioned here that the wood chips of sample A had some wood chips from sample B added by accident. Sample B, however, is still pure. The pmC´s do not seem to stabilise completely in the case of sample B which indicate that the extractions may not be 100% complete. However the increase is only two pmC from the first to the last extraction, so for the purpose of estimating the origin of formic acid this is good enough.

Table 7. Carbon isotope analysis of wood and formic acid from Vasa

Name	Description	Corrected pmC†	Conventional Age	$\delta^{13}C$(‰)‡	PP
A_SPAN1	Wood chips extracted with organic solvents	93.35±0.23	550±20 BP	-24.49±0.27	2.89
A_SPAN2	Wood chips extracted with organic solvents and with an acid-alkali-acid series.	94.41±0.32	460±25 BP	-24.64±0.12	1.79
A_SPAN3	Wood chips extracted with organic solvents and with an acid-alkali-acid series and a series of hot water extractions.	93.56±0.29	535±25 BP	-24.65±0.19	2.71
B_SPAN1	Wood chips extracted with organic solvents	94.50±0.23	455±20 BP	-24.47±0.13	1.73
B_SPAN2	Wood chips extracted with organic solvents and with an acid-alkali-acid series.	95.38±0.24	380±20 BP	-25.69±0.14	0.81
B_SPAN3	Wood chips extracted with organic solvents and with an acid-alkali-acid series and a series of hot water extractions.	96.18±0.26	315±20 BP	-24.42±0.11	0.00*
A_OX	CO_2 from purified HCOOH	91.51±0.25	715 ± 20 BP	-22.19±0.11	4.86
B_OX	CO_2 from purified HCOOH	164.10±0.38	<1955 AD	-24.72±0.26	-

†"Corrected pmC" indicates the percent of modern (1950) carbon, corrected for fractionation using the ^{13}C measurement.
‡ δ ^{13}C includes fractionation due to sample pre-treatment and to AMS as described in section 4.3.1.6.
*This value was used to set the PP scale to zero because it is the most accurate representation of the Vasa wood ^{14}C content available. This sample is pure (not mixed with A) and it has the highest pmC (it has been extracted the most times).

The formic acid isolated from these wood samples was converted to CO_2 and measured by AMS. The results are shown in Table 7 (A_OX and B_OX). The measurement of sample A_OX looks persuasive with a corrected pmC of 91.51. This pmC is almost as high as the pmC for the wood in that sample. Sample B_OX, however, reveals that something is wrong. The pmC for sample B_OX is 164.10, but the maximum value on the pmC scale is 100% which corresponds to the natural atmospheric ^{14}C content in 1950. This sample however contains more than that and not even a post-bomb calibration (1950 to now) can explain a pmC this high convincingly. The conclusion is that the equipment or facilities must be contaminated with something enriched in ^{14}C. It cannot be a contamination with something that has a natural ^{14}C level as this would not be enough to raise the pmC to over 100 %. The first suspicion fell on the $D^{13}COONa$ that had been in contact with the vacuum line so often. This substance is 99 % pure in ^{13}C and it could be that the last 1 % C was enriched in ^{14}C. A test using scintillation counting on this substance showed that the natural HCOONa reference material had the same count as the $D^{13}COONa$ isotope.‡

Another explanation could be that the laboratory where the isolations were performed is contaminated with synthetic isotopes (the facility is "hot"), and that these have contaminated the sample somehow. Since the natural level of ^{14}C is so low (^{14}C:^{12}C ratio; $1{:}10^{12}$), it takes very little ^{14}C to contaminate a sample. One common reason for ^{14}C-contaminated laboratories is biological experiments with ^{14}C-labelled molecules such as $^{14}CO_2$ (from $^{14}CO_3^{2-}$) to investigate the rate of photosynthesis in plants. The problem with "hot" laboratories is well known at AMS facilities such as the one used here. Problems with ^{14}C contamination have been reported for a Danish University.[103]

The ^{14}C results obtained for formic acid, shown in Table 7, were measured on samples purified at Risø National Laboratories in an old plant research department. Although no records of labelling experiments existed, the suspicion fell on these facilities. For this reason part of the formic acid isolation procedure was moved to one of the laboratories of the Danish National Museum in Brede, Denmark. Furthermore, new chemicals were bought for the entire isolation procedure and delivered to the new facility in Brede. The glassware that had to be moved from Risø to Brede was

‡ Measured at Risø National Laboratory for Sustainable Energy, Technical University of Denmark, Radiation Research Division, by Head of Program Sven P. Nielsen and Senior Scientist Xiaolin Hou.

cleaned in a bath made up of concentrated sulphuric acid and hydrogen peroxide (30 %) for several days. No change was made to the isolation procedure itself. The only step that was still performed at Risø was the oxidation of formic acid to carbon dioxide and the isolation thereof in glass ampoules. It should be mentioned that the wood samples, A and B_SPAN, shown in Table 7 have not been in contact with the possibly contaminated facilities at Risø.

After this cleaning, a control experiment was performed to find out if the problem with contamination had been solved. A new bottle of 98 % formic acid was bought and analysed directly by AMS (C_HCOOH). Another sample from the same bottle was subjected to the entire series of isolation procedures (VD1, IE, VD2 and OX) and then analysed as CO_2 by AMS (named C_OX). The results are shown in Table 8.

Table 8. Carbon isotope analysis of formic acid from the Vasa and control experiments

	Sample Description	Corrected pmC†	Conventional Age	$\delta^{13}C$(‰)‡	PP
C_HCOOH	98 % formic acid	0.83±0.10	38460+1070/ -950 BP	-26.29±0.18	99.29
C_OX	CO_2 from formic acid	12.94±0.21	16420±130 BP	-31.49±0.09	86.67
D_OX	Wood from the Vasa	11.70±0.12	17230±80 BP	-28.18±0.20	87.96

†"Corrected pmC" indicates the percent of modern (1950) carbon, corrected for fractionation using the ^{13}C measurement.
‡ $\delta\ ^{13}C$ includes fractionation due to sample pre-treatment and to AMS as described in section 4.3.1.6.

It is seen that the formic acid (C_HCOOH) is 99.29 percent petrochemical (corrected pmC=0.83), which is in good agreement with the product being a petrochemical. The CO_2 that came out of the purification of this formic acid (C_OX) performed in Brede and at Risø, is 86.67 percent petrochemical (pmC=12.94). Thus the carbon went from being 99.29 PP to 86.67 PP after isolation indicating that a contamination corresponding to 12.62 PP (99.29 PP – 86.67 PP=12.62 PP) has appeared during the isolation procedure. The contamination has been reduced drastically from the first situation in which a pmC of 164 was measured for sample B_OX (at least 64 pmC too high). Even though the situation is far from ideal it was decided to analyse a real Vasa sample using this procedure. Formic acid was isolated from a Vasa sample with many salt precipitations on the surface and converted to CO_2, which was then analysed by AMS (D_OX in Table 8). The analysis showed that the sample is 87.96 percent petrochemical (11.70 pmC). If contamination is assumed to be fairly constant from the control experiment (C_OX) to the real Vasa sample (D_OX), then all of the ^{14}C in D_OX would come from the contamination and the formic acid would really be 100 PP. In this interpretation, all of the formic acid is a product of PEG degradation. Another way of viewing the result is by assuming that the ^{14}C contamination is not constant and that the sample from the Vasa (D_OX) has not been contaminated at all. In this scenario, formic acid would have a PP of 87.96, i.e. 87.96 percent of the formic acid in the sample comes from PEG degradation and the rest from the Vasa wood components. The two scenarios considered here define the upper and lower limits of the ^{14}C content that is really in the sample. If the sample has not been contaminated then the formic acid in D_OX is 100 PP, whereas if all the ^{14}C in the sample comes from contamination then the PP is 87.96 PP. Thus the PP of formic acid isolated from D_OX is between 87.96 PP and 100 PP. If this is expressed as $100>PP\geq87.96$ (i.e. PP not equal to 100), then the fact that some formic acid was found in non-impregnated wood is acknowledged (section 4.4.2.).

The ^{13}C shift ($\delta^{13}C$) was reported for all the AMS measurements in Table 6, Table 7 and Table 8. This number is given as a shift (in ‰) relative to the PDB standard (PeeDee Belimnite). In the present case, the $\delta^{13}C$ values represent the sum of fractionation corresponding to natural fractionation and fractionation induced in the sample pre-treatment before measurement on the AMS. Thus these numbers are not very accurate and they should be treated accordingly. They are

all between $\delta^{13}C$ –22 ‰ and –31 ‰. This seems sensible when the $\delta^{13}C$ values reported are compared to numbers from the literature. For example, wood cellulose is reported to have a $\delta^{13}C$ of –20 ‰,. For recent wood this is –25 ‰.[92] Even though no real comparison can be made between the $\delta^{13}C$ measurements given here and natural ^{13}C reservoirs (because of the sample pre-treatment 4.3.1.6), it is positive that the $\delta^{13}C$ values are in the normal range as certain contaminations from the isolation procedure could affect this number. For example if the ^{14}C contamination discussed above was caused by the use of the isotope $D^{13}COONa$ in the laboratory, then the measured $\delta^{13}C$ would have revealed this. The $\delta^{13}C$ numbers are very sensitive to contaminations with isotopes enriched in ^{13}C. Thus the $\delta^{13}C$ values reported here do not reveal any extraordinary contaminations or fractionations.

In summary, it seems likely that the PEG in the Vasa is petrochemical based on the measurements on PEG given in Table 6; the average of the three pmC values given here was used to set the PP scale for 100. The radiocarbon measurement on wood in Table 7 showed that the pmC was 96.18 for B_SPAN and this was used to set the zero of the PP scale. Control experiments showed that a small ^{14}C contamination is still added to the sample when it is isolated in spite of the efforts made to avoid this. The sample that was measured from the Vasa (D_OX) contained formic acid with a PP of 87.96. This measurement combined with the control experiment, allows us to say that the formic acid isolated from the Vasa sample (D_OX) has a PP beween $100>PP\geq87.96$.

Thus most of the formic acid in the sample is petrochemical and hence originates from PEG. This means that PEG degradation is a reality. It is true that some formic acid originates from the wood components, but some of the formic acid is petrochemical and that can only be explained by PEG degradation.

The formic acid content in different samples was discussed in section 4.4.2. Using average formic acid concentrations in wood and an estimate of the PEG content it was calculated that it would take 76000 years to transform all of the PEG in the ship to formic acid. If the measured PP of 87.96 for the Vasa is assumed to represent the true fraction of petrochemical/recent formic acid then the real content of PEG produced formic acid is 0.8796 * 0.031 % = 0.027 %. This corresponds to 2.96 mmole EG monomer per kg wood. For the Vasa this corresponds to 0.053% of the total EG monomers in the ship and the rate of degradation then becomes 0.0012% per year which gives the ship 86000 years before all EG monomers are gone. This is slightly higher than the 76000 years predicted in section 4.4.2. This is a silly number, of course, since it is so high and it almost contradicts the fact that PEG degradation has taken place as shown by the PP between 100 and 87.96. One explanation could be that the situation is not static as assumed here but dynamic. This is an interesting thought in light of the finding that $100>PP\geq87.96$ is true for formic acid in a sample from the Vasa. Continuous evaporation of formic acid from the wood with the named formic acid PP would require constant formation of both petrochemical and wood-based formic acid. Either PEG degradation is taking place like this, continually, along with a reaction that leads to formic acid from wood (dynamic interpretation), or only very little PEG degradation has taken place since the onset of conservation 46 years ago (static interpretation). In the static interpretation PEG degradation could have taken place during the spray treatment of the ship and during melting of the surplus PEG from the wood surface, as discussed in a previous section.

What can be said about PEG degradation in the Vasa based on the ^{14}C measurement is that at least some PEG degradation has taken place at some point since the onset of conservation. It is

not known if PEG degradation is taking place continually; if this is the case then formic acid is also released from the wood continually.

4.5.3 Conclusions

It seems likely that the PEG in the Vasa is petrochemical since three samples of PEG leftover from the conservation had ^{14}C contents close to zero.

The ^{14}C content of wood from the Vasa was in agreement with the age of the Vasa (322 BP).

The extent of contamination of sample formic acid with foreign ^{14}C in the isolation procedure has been reduced substantially, but not removed.

It could be concluded that the PP of the formic acid in a sample from the Vasa was $100>PP\geq87.96$. In spite of the possible contamination, it is still safe to conclude that most of the formic acid in the sample is petrochemical and thus originates from PEG. Thus at least some PEG degradation has taken place in the Vasa at some point since the onset of conservation. If formic acid is evaporating from the wood continually then PEG degradation is an ongoing process, and so is the release of formic acid from the wood. *It should be kept in mind that these conclusions rely on a single measurement of contaminated formic acid from a single piece of Vasa wood and some very low formic acid concentrations. Thus no far-reaching decisions regarding future treatment of the Vasa should rely on this measurement alone.*

4.6 Conclusions

A method for determining formic acid concentration was developed for SPME GC-MS.

A method for determining the $^{12/13}C$-fraction in mixtures of natural $H^{12}COOH$ formic acid and the isotope $D^{13}COOH$ was developed for SPME GC-MS. It was also possible to use a GC-MS gas analysis for the determination of the ratio $^{12/13}CO_2$.

A method for isolating formic acid from PEG-impregnated archaeological wood for radiocarbon analysis was developed and tested. The procedure causes a slight contamination of the samples with foreign carbon, but only very little.

The formic acid content of four different ships impregnated with PEG like the Vasa was measured using an SPME GC-MS method. The average formic acid content of the Vasa was 0.031 % by weight.

The ^{14}C content of formic acid isolated from a sample from the Vasa was $100>PP\geq87.96$ percent petrochemical. Thus some PEG degradation has taken place in the Vasa.

5 Summary

Part of the present work relates to characterisation of the PEG-impregnation in the Vasa and the Skuldelev Viking ships. This was carried out using a range of different analytical techniques. PEG extractions and ATR FT-IR analysis showed that large amounts of PEG is in the surface layers of the wood and smaller amounts of PEG are found deeper inside the wood of the Vasa and the Skuldelev Viking ships. It also showed that PEG is in the parts of the wood that are more degraded and porous. For the Vasa, MALDI-TOF MS showed that sound wood in particular is too dense for the large PEG 4000 molecule to migrate very deep into it while PEG 1500 and PEG 600 are present at all depths. PEG with a tailing molecular weight distribution (molecular weight < 2000 g/mol) was detected in a sample from the Skuldelev Viking ships both with MALDI-TOF MS and SEC. This could be interpreted as a product of PEG 4000 degradation since PEG 4000 was the only PEG used on these ships. Furthermore, maintenance of the Skuldelev ships included melting PEG into the wood surface, which could have led to PEG degradation. On the other hand tailing molecular weight distributions were observed in some of the batches of PEG that were left unused after the conservation of the Vasa. This makes it harder to use tailing molecular weights as an indication of PEG degradation in the Vasa timbers and in general. Satellite peaks have been discussed frequently in relation to the Vasa and the Skuldelev Viking ships. Assignments were proposed in the present work that suggest that the satellite peaks are artefacts of the MALDI-TOF MS technique. Thus using this technique to detect PEG reaction products may be problematic.

The overall impression of the state of PEG in the Vasa and the Skuldelev Viking ships is that the PEG looks fairly sound. Some tailing was observed in the PEG molecular weight from the Skuldelev ships, but none of the evidence considered here suggests that this is the result of an ongoing degradation process.

Accelerated ageing experiments were performed in an attempt to describe the reactions involved in PEG degradation. It was demonstrated that oxidation dominates the degradation of TEG at 70 °C, and that formic acid is the primary product of the reaction. Mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol were also detected in the process using GC-MS. PEG 1500 undergoes a similar oxidation. Satellite peaks were observed in the ESI MS, which were assigned to a PEG formate and, tentatively, to an in-chain ester. TEG thermo-oxidation was inhibited by small quantities of a group of components. These include KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2, $CuSO_4$, fresh oak wood sawdust and a scraping from the Vasa containing gypsum. It was speculated that the antioxidant action of the salts was due to catalytic removal of a TEG hydroperoxide. The antioxidant action of wood could act in a way similar to the known phenol antioxidants. Here removal of radicals leads to polymerisation of the phenol antioxidants. The scraping from the Vasa containing gypsum probably also contains wood.

Overall, accelerated ageing showed that formic acid is the end product of PEG degradation. Thus formic acid should be in objects where PEG degradation has taken place. Furthermore wood and a range of salts slow down TEG thermo-oxidation. This might suggest that PEG in a wood matrix is less prone to oxidation than previously anticipated.

An attempt was made to take advantage of formic acid as a marker for PEG degradation in PEG-treated wooden shipwrecks. The concentration of formic acid was determined in samples from the Vasa, the Oberländer boat, the Skuldelev Viking ships and the Bremen cog; none of them

contained over 1 % formic acid by weight in the wood. The formic acid contents were equal to or slightly elevated compared to archaeological wood that has not received PEG-treatment (measured for the Bremen cog and the Skuldelev ships). If these formic acid contents were translated into PEG degradation since the onset of conservation, assuming that all the formic acid originates from PEG, then it would take approximately 70000 years to degrade the PEG that is estimated to be in the ships (the Vasa or the Skuldelev, in the example). Formic acid was isolated from the Vasa and oxidized to CO_2. Radiocarbon analysis of this CO_2 showed that the formic acid in the Vasa is between 100 and 88 percent petrochemical, where 0 means that it originates from Vasa wood and 100 means that it originates from PEG. Thus formic acid in the Vasa sample is mostly petrochemical which means that it originates from PEG. It can be concluded that at least some PEG degradation has taken place in the Vasa at some point since the onset of conservation.

It was shown that formic acid is in the Vasa, the Skuldelev ships and in other PEG-treated wooden objects. Radiocarbon analysis showed that formic acid in a Vasa sample mainly originated from PEG. This demonstrates that PEG degradation has taken place, however the extent of degradation may be limited.

5.1.1 Outlook

It could not be shown definitively with the characterisation experiments if PEG degradation had taken place in the Vasa and the Skuldelev ships. Satellite peaks were observed in MALDI-TOF MS spectra of PEG extracted from the Vasa and the Skuldelev ships. However, the most realistic assignments are also considered as artefacts of the MALDI technique. Tailing PEG molecular weights were observed in PEG that had been saved from the conservation of the Vasa. It was also discussed that tailing molecular weight distributions could result from the mixing of several batches of PEG with slightly different molecular weights. Thus tailing molecular weight distributions cannot be taken as an unambiguous sign that PEG degradation has taken place in the ships. The PEG molecular weight distributions mostly looked sound in the Vasa and the Skuldelev ships. However, in a Skuldelev sample tailing molecular weight distributions were observed. This ship has received treatment with hot air, which could lead to PEG degradation, but the tailing molecular weight distribution does not prove PEG degradation. Thus future work should not focus on either PEG molecular weight distribution or satellite peaks in MALDI-TOF MS as indications of PEG degradation.

The accelerated ageing experiments, among other things, showed that formic acid is a product of TEG degradation. A mechanism for the oxidative degradation was suggested. There are many investigations of PEG degradation the literature. The mechanisms presented in the literature are often in disagreement with one another. Thus further work on accelerated ageing of PEG should to focus on new approaches to the problem. For example the use of ESR (Electron Spin Resonance) has not yet been explored in this context even though the reaction is claimed to be a radical reaction by many. However, it could be argued that further mechanistic specificities are of only moderate interest to the Vasa or to PEG-impregnated wooden shipwrecks in general. Several components had an effect on the rate of degradation of PEG; it was especially surprising to find that wood slowed down the degradation. This suggests that future work on stability and degradation of components in a matrix like the Vasa should relate to several compounds. Since different components in the matrix appear to interact, it may be dangerous to focus on a single substance such as PEG. Lignin, cellulose, hemicellulose and all the other components in the matrix could be degrading just as well as PEG and they could be affecting the stability of one another. Measurements that could describe the entire matrix at room temperature would be relevant. Thus, further experiments could include monitoring the respiration of entire pieces of wood from the Vasa in a small headspace (respirometry). At least the oxidative processes could be

monitored this way. Some salts had an antioxidant effect. It could be interesting to investigate the mechanism behind this action, but mostly for salts that are found within the wood matrix.

The only investigation that pointed at degradation of PEG was the measurement of formic acid concentration and radiocarbon analysis of formic acid in the Vasa. It showed that PEG degradation had taken place to some extent at some point in time since conservation was initiated for the Vasa. The extent of degradation could not be determined accurately because of a contamination in the purification steps and because formic acid may be evaporating from the wood. It would be relevant to improve the technique for isolating formic acid in order to remove the contamination. It is likely that moving the vacuum-line to a different facility would solve the problem. If removing the ^{14}C contamination could be accomplished, then more samples should be analysed. At least a few samples from the Vasa and the Skuldelev ships should be analysed. If these measurements also showed that the formic acid is mostly petrochemical, then it would be relevant to look further into formic acid concentrations and evaporation from wood. It might be possible to investigate evaporation from the surface of a Vasa sample by SPME GC-MS headspace analysis of formic acid in an airtight plastic bag with a well-defined volume containing a block of sample wood.

5.1.2 Overall conclusion

Some of the experiments that were carried out here could benefit from supplementation. However, if the results are summed up in terms of PEG degradation in the Vasa and the Skuldelev ships, it seems most likely that degradation has taken place but to a small extent only. The heating of PEG in the ships seems like a plausible cause of the degradation and thus refraining from using heat could prevent further degradation of PEG.

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Appendix 1

Article I:
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Characterisation of the polyethylene glycol impregnation of the Swedish warship Vasa and one of the Danish Skuldelev Viking ships

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Abstract

The Swedish 17th century warship Vasa and the Danish Skuldelev Viking ships from the 11th century were impregnated with polyethylene glycol (PEG) in the 1960s. The molecular weight, amount and integrity of this PEG were investigated at a range of depths below the wood surface. Large amounts of PEG could be extracted from degraded parts of the ships but hardly any from sound parts. Mass spectrometry showed that PEG 4000 is present only in the surface layers of the wood, PEG 1500 and PEG 600 are present at all depths of the wood that has been treated with it. Low molecular weight PEG was detected in one of the Skuldelev ships by mass spectrometry and Size Exclusion Chromatography (SEC), it is argued that this is due to degradation of PEG 4000. SEC also showed that PEG 600 is the major PEG component in the Vasa which makes this particular object sensitive to changes in air humidity since PEG 600 is hygroscopic.

Keywords: Waterlogged wood; Polyethylene glycol; PEG; Distribution; Characterisation

1. Introduction

The stability of PEG (polyethylene glycol) and its ability to penetrate waterlogged wood is of great importance in conservation. Waterlogged wood will bend and crack if it is dried without preservation. To avoid this, water in the wood must be replaced by something that does not evaporate. PEG has proven useful for this purpose several times since it was put into use in the 1960s (Håfors, 1999, 1990; Hoffmann et al., 2004; Jensen et al., 2002; Morén and Centerwall, 1960; Seborg and Inverari, 1962). Normally the artifact is soaked with an aqueous PEG solution, either by submerging it into such a solution or by spraying the solution onto the artifact, this allows the PEG to diffuse into the wood. It takes 10–20 years to impregnate an average size ship timber at room temperature. The Batavia in Australia, the Bremen Cog in Germany, the Skuldelev ships in Denmark and the Vasa in Sweden are examples of PEG impregnated wooden shipwrecks on exhibition today. In Portsmouth England, the Mary Rose is currently receiving PEG treatment.

The Vasa was salvaged in the Stockholm harbour in 1961 after 333 years on the seabed. It was impregnated with aqueous solutions of PEG 600, PEG 1500 and PEG 4000. From 1962 to 1965, PEG 4000 and PEG 1500 were applied manually to the parts of the ship that seemed to need it. In 1965 an automatic spraying system was started, it covered the entire ship in a mist of PEG 1500 solution. From 1971 until the automatic system was stopped in 1979, PEG 600 was used. Between 1979 and 1991 PEG 600 and PEG 4000 was applied manually, excess PEG 4000 was melted off the wood surface

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using hot air blowers (Håfors, 2001). When treatment started in 1962 Vasa was the first object of its size to receive PEG impregnation. At this time the excavation of the Skuldelev ships in Denmark was almost complete. The Skuldelev ships were sunk sometime during 1070–1090 AD, at Skuldelev, Denmark, to block the narrow channel. When the timbers had been excavated they were impregnated by submersion into tanks containing an aqueous PEG 4000 solution (Jensen et al., 2002). During impregnation the PEG uptake in the timbers was monitored for both the Vasa (Håfors, 1990) and the Skuldelev ships (Jensen et al., 2002) using different techniques. However, these measurements were not meant to give detailed information about PEG concentration as a function of depth below the wood surface neither for the Vasa nor Skuldelev. In this study the total PEG content as well as the content of the various PEG types used, is measured as a function of depth below the wood surface.

A series of observations have recently caused concern at the Vasa museum in Stockholm. These include acid formation on the wood threatening to hydrolyse wood cellulose fibers (Sandström et al., 2003), a suspicion that the timbers are getting softer and outbursts of colored salts in patches on the wood surfaces. These are some of the reasons for investigating PEG in the Vasa and the Skuldelev ships. Many aspects of PEG degradation have been studied. It has been shown that a number of factors affect the degradation as for example different kinds of electromagnetic radiation (Decker and Marchal, 1974; Morlat and Gardette, 2001, 2003; Vijayalakshmi et al., 2005a,b), electromagnetic radiation or heat in combination with the presence of salts (Costa et al., 1992; Gjurova et al., 1997, 1999; Glastrup and Padfield, 1993; Kaczmarek, 1996; Kaczmarek et al., 1999, 1996, 2001; Kaczmarek and Rabek, 1997; Kaminska et al., 1999; Lloyd, 1963; Rabek et al., 1992), unassisted pyrolysis and oxidation has also been investigated (Bilz et al., 1994; Dulog and Storck, 1966; Evetts et al., 1995; Fares et al., 1994; Geymayer et al., 1991; Glastrup, 1996; Goglev and Neiman, 1967; Grassie and Mendoza, 1984; Han et al., 1995, 1997; Hoffmann et al., 1984; Kaczmarek et al., 2000; Lattimer, 2000; Madorsky and Straus, 1959; Mcgary, 1960; Mkhatresh and Heatley, 2002, 2004; Neto et al., 2002; Padfield et al., 1990; Scheirs et al., 1991; Voorhees et al., 1994; Yang et al., 1996). Oxidation of PEG can take place at relatively low temperatures (Bilz et al., 1994; Geymayer et al., 1991; Han et al., 1995; Padfield et al., 1990; Scheirs et al., 1991). Formic acid and formates of PEG have been suggested as major products of oxidative PEG degradation (Geymayer et al., 1991; Glastrup, 1996; Han et al., 1995, 1997; Yang et al., 1996). Formic acid produced by PEG degradation could contribute to the low pH on the Vasa timbers. Lowered PEG molecular weight, caused by degradation is another problem. If the molecular weight gets too low, PEG turns into a liquid. Low molecular weight PEG is more hygroscopic than high molecular weight PEG. An ultimate consequence of these factors could be that impregnation started to seep out of the timbers. No investigation has been conducted to assess the integrity of PEG in the Vasa or the Skuldelev ships.

2. Materials and methods

2.1. Samples

Four samples were acquired; three samples from the inside of the Vasa planking and one from the keelson of the Skuldelev 2 Viking ship. The samples from Vasa were taken at three different locations, one where the wood seemed in good condition (designated V_G), one that looked very degraded (designated V_D) and one from a location with intermediately degraded wood (designated V_M). The sample from Skuldelev 2 (designated S) was in good condition. The locations where V_D and V_M were taken have been treated with an aqueous carbonate buffer in an attempt to neutralize the acid formed in the timbers (Sandström et al., 2003). All samples are PEG treated oak, PEG 600, 1500 and 4000 for the Vasa (Håfors, 2001) and PEG 4000 for the Skuldelev ships (Jensen et al., 2002). The samples were cylinders, approximately 5 cm long and 2.5 cm in diameter, taken at right angles to the wood surface (Vasa's interior) using a plug centre bit (Fig. 1). The plugs were sliced into discs, approximately 5 mm thick, on a fretsaw parallel to the grain and to the surface. In this way the saw blade was kept in one PEG concentration plane to prevent PEG contamination between different layers.

2.2. Extractions

Soxhlet extraction was carried out on samples from each wood disc. This gave information on how much PEG could be extracted from the wood as a function of depth below the surface, and it yielded PEG extracts for further analysis. Wood chips were cut from the sample discs with a pair of nippers after the possibly contaminated edges of the disc had been removed. The nippers were cleaned in ethanol in an ultrasonic bath and dried between every sample disc. A new extraction thimble (10 × 50 mm) was filled with wood chips (0.4–1.1 g) and weighed accurately before it was placed

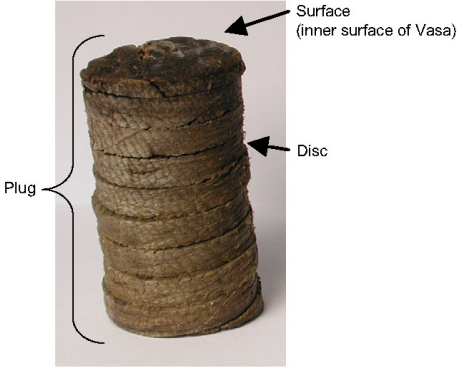

Fig. 1. A sliced plug from the Vasa (V_G). The plug is about 5 cm long, 2.5 cm in diameter and a disc is approximately 5 mm thick.

in the soxhlet. The soxhlet was mounted on a 50 mL round bottomed flask containing 30 mL chloroform and a magnetic stirrer. Chloroform was chosen in preference of methanol because it extracted as much PEG from the wood as methanol, but less resins (based on Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis and weight loss of the wood chips). The flask was in contact with sand on a heating plate. The cooler was connected to a manifold that allowed swapping between vacuum and nitrogen gas. Air was removed from the setup by vacuum and replaced by nitrogen; this was repeated 10 times before the extraction was started. During extraction the system was pressurised slightly with nitrogen. Teflon seals were used in all joints. The entire setup was wrapped in aluminum foil to exclude light. The temperature on the heating plate was 150 °C, which resulted in approximately 70 drainings per hour of the soxhlet. All samples were extracted for 3 h since longer extractions resulted in no further weight loss of the wood chips. After gravity filtration through a dense filter paper, the solvent was evaporated from the extract at room temperature under a stream of nitrogen. The resulting light yellow paste was stored in vacuum until it was measured on the mass spectrometer a week later. Eight thimbles, two from each sample plug, were placed in vacuum and weighed daily; the weights of these thimbles had all stabilized after five days. Based on this information it was decided to place all thimbles in vacuum for seven days before weighing them. To make sure that the extraction procedure did not affect PEG, fresh PEG 4000 was extracted using the same procedure. MALDI-TOF mass spectra of the extract were identical to MALDI-TOF mass spectra of PEG 4000 that had not been extracted, thus the extraction procedure itself did not affect PEG.

2.3. Mass spectrometry

The extracts were analysed by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). Spectra were obtained using a dihydroxybenzoic acid (DHB)/NaCl matrix in a sample:matrix ratio of 1:1. The matrix consisted of 10% NaCl solution (1 g L^{-1} in H_2O), and 90% DHB solution (30 g L^{-1} in tetrahydofurane). The sample/matrix mixture was dried and analysed on a BRUKER Reflex IV using a UV laser (337 nm). Spectra were recorded at both 30% and 45% laser power.

2.4. Size Exclusion Chromatography

The content of the different polyethylene glycols, was determined quantitatively using Size Exclusion Chromatography (SEC). The analysis was set up on a Shimadzu HPLC equipped with refractive index detector. A combination of two columns was used, a TOSOH TSKgel G 3000 PW and a TSKgel G 2500 PW with an inner diameter of 7.5 mm, a length of 30 cm and particle size of 10 μm. Separation of PEG 600, 1500 and 4000 was achieved with these columns. When the samples were analysed, an external standard mixture was measured regularly. It contained appropriate amounts of the PEG 620, PEG 1470 and PEG 4020 standards dissolved in the mobile phase. The calculations of PEG concentrations were based on these standards. 20–30 mg of the sample extract was weighed accurately and dissolved in 4 mL of mobile phase. The mobile phase was 25 mM NaCl in methanol:water (5:95). The flow was 1 mL min^{-1} and 100 μL sample was injected.

3. Results and discussion

3.1. Extractions

The weight loss of the wood chips after extraction, in percent of the start weight, is proportional to the amount of PEG in the sample. This percentage is plotted versus depth below the wood surface (distance from surface to midpoint of disc) in Fig. 2. It is seen that in the degraded and the intermediately degraded Vasa samples (V_D and V_M) PEG constitutes about half the total sample weight, the PEG content is almost constant throughout the depth of these samples. In the sound Vasa sample and the Skuldelev sample which

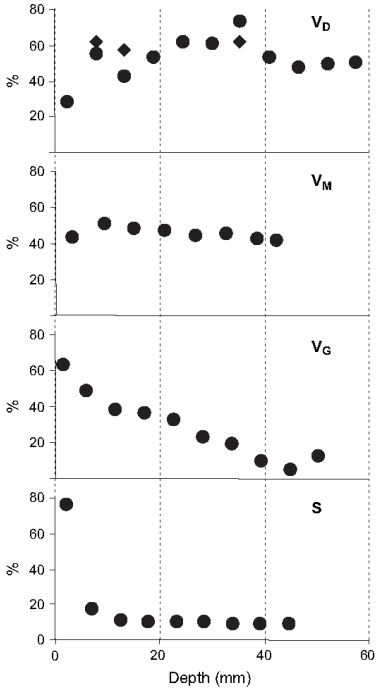

Fig. 2. Plots of % (the percentage extracted) versus depth are shown for all four samples. The percentage extracted (%) is the weight loss of the wood chips after extraction in percent of the start weight of the wood chips. Depth is the position of the midpoint of a disc relative to the surface. From top: V_D, V_M, V_G and S. Diamonds are reruns (new wood chips from same disc).

were both very dense (V_G and S), the PEG content is high in the outermost layers (60–80%) but drops to very low concentrations deeper inside the wood. These PEG concentration profiles are related to the condition of the wood before impregnation. V_D and V_M are porous in the full sample depth, V_G and S are porous in the surface layers but the porosity decreases closer to the core (degradation starts at the surface and works its way into the core). This trend in hardness was observed when the sample discs were cut with the nippers. Thus the more degraded the wood is before impregnation the more PEG it will accommodate.

As mentioned some parts of the Vasa are acidic (Sandström et al., 2002), the problem is alleviated by neutralization with a carbonate buffer (Sandström et al., 2003) while other strategies for neutralization are being investigated (Giorgi et al., 2005). The parts of the Vasa where V_D and V_M were taken has been subjected to buffer treatment, this is not the case for the other two samples (V_G and S). It is seen from Fig. 2 that V_D and V_M contain slightly less PEG in the surface than in the rest of the sample. One would expect more PEG, or at least the same amount of PEG, in the surface layers compared to the rest of the sample, as seen for V_G and S. Thus, treatment of the wood with aqueous carbonate buffer removes PEG from the surface layers.

3.2. Mass spectrometry

MALDI-TOF mass spectra recorded on the extracts of the wood discs are shown in Figs. 3–5. Peaks corresponding to singly charged ions of PEG ($[(PEG)Na]^+$) dominate the spectra. The peaks are spaced by 44 mass units and they appear, mostly, as Gaussian distributions.

The MALDI-TOF mass spectrum of the outer 3 mm of V_G show ions distributed around *m/z* 4000, *m/z* 1500 and *m/z* 600 (Fig. 3). This is in agreement with the presence of PEG 4000, PEG 1500 and PEG 600 in the sample. Deeper inside the wood, from 3 to 53 mm, ions corresponding to PEG 600 and PEG 1500 are seen but no PEG 4000. Thus PEG 4000 is unable to enter the wood, but both PEG 1500 and PEG 600 are able to do so. MALDI-TOF MS recordings on V_M extracts show PEG 600 and PEG 1500 at all depths of the sample and PEG 4000 in the surface layers only, as for V_G (data not shown). MALDI-TOF MS recordings show that PEG 1500 and PEG 600 are present at all depths of V_D (Fig. 4). In that respect V_D is like the other Vasa samples, however, no PEG 4000 was found at any depth of the sample. Instead PEG with a molecular weight around 2500 g/mol was found from 5 to 22 mm below the wood surface. There is no record of PEG 2500 treatment of the Vasa but the PEG 2500 signal does not look like a product of degradation because the dispersity is low and the distribution is symmetric. It is not possible to give a definitive explanation for the absence of PEG 4000 and presence of PEG 2500, however, the PEG 2500 could originate from a batch of PEG that contained PEG 2500 instead of PEG 4000. This would explain the absence of PEG 4000 in the surface layers and the presence of PEG 2500 down to 22 mm. If this PEG 2500 was applied manually

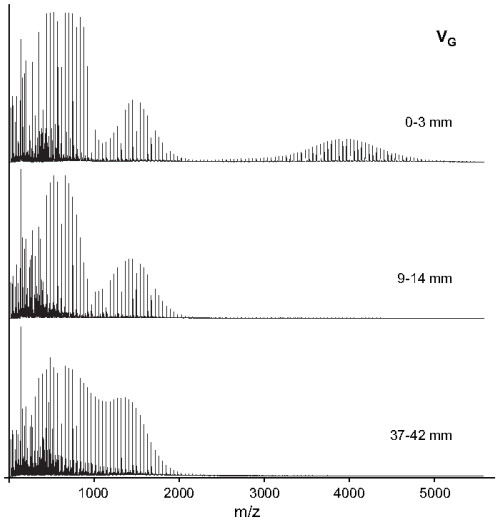

Fig. 3. MALDI-TOF mass spectra recorded on the extracts of the wood discs from V_G (from top): the outermost 3 mm, 9–14 mm below the wood surface and 37–42 mm below the wood surface. The scale on the *x*-axis is the same for all three inserts, the *y*-axis have arbitrary scales.

in the very beginning of the treatment from 1962 to 1965 it could have been washed out of the surface layers later when the automatic spraying system covered the Vasa in a mist of aqueous PEG 1500 and later PEG 600. This would explain the absence of PEG 2500 and presence of PEG 1500 and PEG 600 in the surface layers.

MALDI-TOF mass spectra recorded on the Skuldelev sample are shown in Fig. 5. This ship was treated with PEG 4000 only, PEG 4000 is detected between 0 and 9 mm from the wood surface. Besides PEG 4000 a broad and asymmetric distribution of PEG at masses less than about 2000 g/mol is observed from the surface and down to 36 mm. As for the Vasa samples, low molecular weight PEG has migrated deeper into the Skuldelev sample than has the PEG 4000. However, the finding of low molecular weight PEG (molecular weight < 2000 g/mol) was unexpected since the Skuldelev ships were treated with PEG 4000 only. One explanation could be oxidative degradation of PEG 4000 which has been shown to produce low molecular weight PEG with asymmetric molecular weight distribution (Geymayer et al., 1991; Han et al., 1997; Padfield et al., 1990). This explanation is plausible since the Skuldelev ships have had fresh PEG 4000 melted into the wood surface once every year for approximately 18 years using hot air blowers according to the Conservator in charge of maintenance at the Viking Ship Museum in Roskilde, Anette Hjelm Petersen. Furthermore the original impregnation took place in tanks containing a warm PEG solution. Similar conditions have led to PEG degradation in other cases (Padfield et al., 1990).

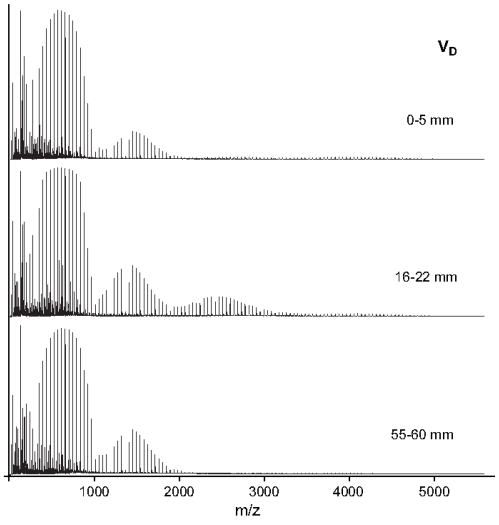

Fig. 4. MALDI-TOF mass spectra recorded on the extracts of the wood discs from V_D (from top): the outermost 5 mm, 16–22 mm below the wood surface and 55–60 mm below the surface. The scale on the *x*-axis is the same for all three inserts, the *y*-axis have arbitrary scales.

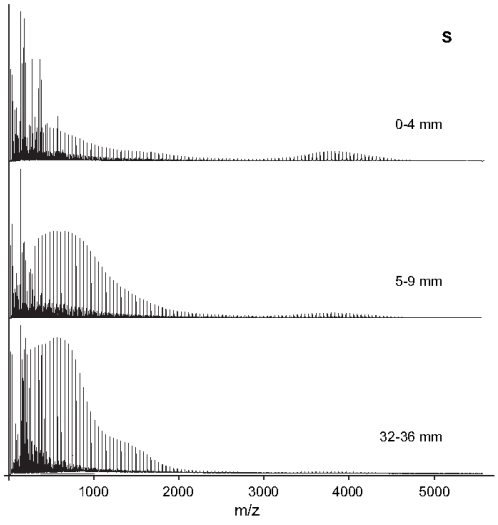

Fig. 5. MALDI-TOF mass spectra recorded on the extracts of the wood discs from S (from top): the outermost 4 mm, 5–9 mm below the wood surface and 32–36 mm below the surface. The scale on the *x*-axis is the same for all three inserts, the *y*-axis have arbitrary scales.

From MALDI-TOF analysis it can be said that particularly sound wood is too dense for PEG 4000 to enter since this was found in surface layers only. It can also be said that PEG 600 and PEG 1500 are able to migrate very deep into even very dense wood since these PEG types were detected at all depths. According to the extraction experiments the amount of PEG in the core of sound wood is limited. On the other hand, the mass spectra show that the PEG that is present here, is PEG 600 and PEG 1500. Thus PEG 600 and PEG 1500 are able to enter sound wood, but sound wood can only accommodate very little PEG, hence the low concentrations.

3.3. Size Exclusion Chromatography

The concentration of the individual PEG types in the wood was determined using Size Exclusion Chromatography (SEC) on the extracts from the wood discs. In Fig. 6 this is plotted, as weight percent PEG in wood, versus depth below the wood surface. In V_D the PEG 600 content is about 10% at the surface, it gradually increases to about 20% in the core. The PEG 1500 and PEG 2500 contents are low (<4%) between 0 and 20 mm and between 40 and 60 mm; between 20 and 40 mm their concentrations peak at approximately 8%. The explanation presented for the MALDI-TOF MS result of this sample can account for the SEC result too. The general idea is that one PEG type starts to wash out of the wood surface when treatment with this is followed by treatment with another PEG type in solution. The only PEG type that is not washed out is the one that is applied last. For V_D, PEG 2500 could have been applied in the beginning of the treatment migrating down to 30 mm below the surface. Later, aqueous PEG 1500 was sprayed on automatically removing some of the PEG 2500 from the surface layers but leaving some behind deeper in the wood (20–40 mm). When the PEG 1500 was changed for PEG 600, some of the surface PEG 1500 was washed out as was PEG 2500. This would explain why the PEG 1500 and PEG 2500 seem to peak between 20 and 40 mm but are absent from the outer layers (0–20 mm), while the PEG 600 concentration is high throughout the depth of the sample. V_M contains 5% to 10% PEG 600, the same amount of PEG 1500 but hardly any PEG 4000 (2%). There is a slight tendency for the concentrations of PEG 1500 and PEG 4000 to peak at 18 mm and for the PEG 600 to be high all the way through the sample as discussed above, but here the tendency is moderate. The PEG contents of V_G all decrease with increasing depth as seen in the extraction experiments for this sample. 38% PEG 600, 8% PEG 1500 and 9% PEG 4000 were found in the surface disc, deeper than 35 mm PEG could only be detected by MALDI-TOF MS. These concentration profiles are not as clear as they are for V_D because the total PEG content decreases with increasing depth. Therefore, these results are not good enough to tell if the sequence of PEG application already described can account for this sample too.

It is interesting to note that the Vasa timbers seem to contain more PEG of lower molecular weight than PEG of higher molecular weight at most depths of all samples. Whether this is due to longer time of exposure of the low molecular weight PEG compared to the other PEG types applied or due to

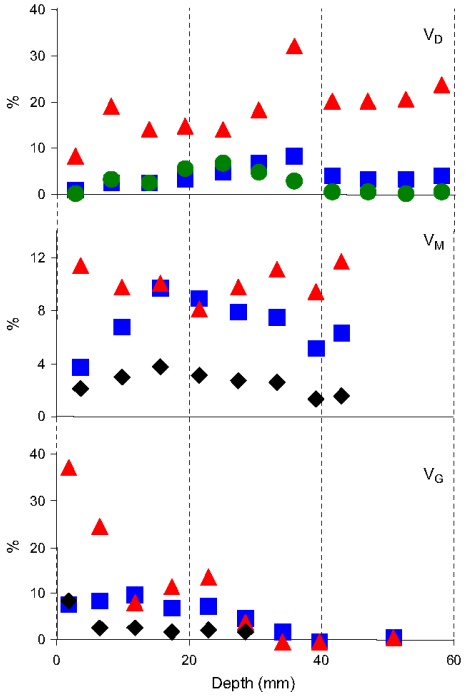

Fig. 6. SEC results from the extracts of the discs from V_D (top), V_M (middle) and V_G (bottom). The *x*-axis show depth below wood surface in millimetre, the *y*-axis show the PEG concentration in weight percent in the wood. Symbols denote PEG molecular size: triangles, PEG 600; squares, PEG 1500; dots, PEG 2500; diamonds, PEG 4000.

effects of wood structure is hard to say. PEG 600 is the major constituent of the PEG that is in the Vasa. Since low molecular weight PEG is hygroscopic and present in large quantities in the Vasa, this ship is particularly sensitive to changes in air humidity. A small change will result in large amounts of water moving in or out of the timbers. Another detail to consider is that PEG is liquid at molecular weights below 600–800 g/mol at room temperature. One could fear that these effects in combination would result in PEG seeping out of the wood.

The concentration of PEG 600, PEG 1500 and PEG 4000 found by SEC should sum up to the same amount as found by extraction if the extract contained PEG 600, PEG 1500 and PEG 4000 only. However, this is not the case. The sum is lower than the concentration found by extraction but the difference between the two values (weight percent PEG in wood found by extraction – sum of weight percent PEG 600, PEG 1500 and PEG 4000 found by SEC) are quite constant within one sample plug (quite low standard deviation (σ)). The average difference over a sample plug is 19% w/w ($\sigma = 5.6\%$ w/w) for V_D, 18% w/w ($\sigma = 2.8\%$ w/w) for V_M and 4.5% w/w ($\sigma = 3.1\%$ w/w) for V_G. The difference between the concentration profiles (concentration versus depth) is big (except for V_G) but the standard deviation is not. This means that the extraction procedure removes a fairly constant amount of components other than PEG 4000, PEG 1500 and PEG 600 from the individual wood discs within one plug. Since this fraction is fairly constant (quite low σ) within one sample plug, the PEG concentration profiles given in Fig. 2 have the right shape nevertheless.

Only the outer two discs of S yielded enough extract for SEC analysis. The surface disc (0–4 mm) contains 63% PEG 4000, it was not possible to detect any low molecular signal in this chromatogram (data not shown). Fig. 7 shows SEC analysis of the second disc (4–9 mm), it is not possible to deduce the PEG 4000 (at retention time 12.85 min) concentration accurately since this signal is overlapped by the low molecular weight (molecular weight < 2000 g/mol) fraction between retention time 13.74 and 18.41 min. If this is attempted anyway as indicated in Fig. 7, the PEG concentrations in the wood are found to be 2% PEG 4000 and 2% low molecular weight PEG. If these 2% low molecular weight PEG is a product of PEG 4000 degradation, the extent of degradation is significant. The low molecular fraction is not necessarily formed from PEG 4000 in the second disc exclusively; it could also have migrated from the first disc that contains large amounts of PEG 4000. A degradation that results in 2% low molecular weight PEG in approximately 18 years may not sound like a fast reaction but if the ships should last hundreds of years, it could become problematic nonetheless.

4. Conclusions

Extraction experiments showed that degraded wood had high contents of PEG at all depths below the wood surface. Sound wood contained high amounts of PEG in the surface layers but little or no PEG in the core. Thus, PEG is found in the parts of the wood that are more degraded and porous. It also seems likely

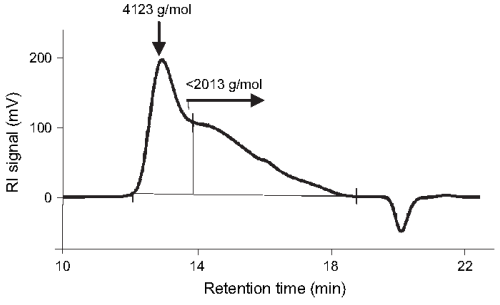

Fig. 7. Size exclusion chromatogram corresponding to the extract from sample S 4–9 mm below the wood surface. The *y*-axis show the refractive index signal from the detector, the *x*-axis show retention time in minutes. The peak at retention time 12.85 min corresponds to PEG 4000 the low molecular weight PEG (<2000 g/mol) is seen between 13.74 and 18.41 min.

that neutralization with aqueous buffer causes removal of PEG from the surface layers of the timbers. Clearly these effects should be taken into consideration before deciding to neutralize PEG treated wooden materials. Mass spectrometry showed that sound wood in particular is too dense for the PEG 4000 molecule to migrate very deep into it. The PEG 1500 and PEG 600 are present at all depths of all samples that have been treated with these PEG types. Thus, even though sound wood only accommodates very little PEG in the core, the PEG that is there consists of PEG 1500 and PEG 600. PEG with a molecular weight of 2500 g/mol was detected in one sample from the Vasa, the origin of this PEG is uncertain. However, it seems likely that a batch of PEG used on the Vasa contained PEG 2500 instead of PEG 4000. Low molecular weight PEG (molecular weight < 2000 g/mol) was detected in the Skuldelev sample both with MALDI-TOF MS and SEC. This may be a product of PEG 4000 degradation since PEG 4000 was the only PEG used on these ships. It is not possible to tell if such a low molecular fraction is present in the Vasa samples since it may be hidden beneath the more intense PEG 1500 and PEG 600 signals. Thus if the low molecular weight PEG is in fact a sign of degradation, then degradation has taken place in the Skuldelev sample and it may or may not have taken place in the Vasa. These findings prompted us to initiate further investigations concerning the extent of degradation and the mechanisms involved. Identification of products formed under accelerated ageing conditions is ongoing in our laboratory and so is the search for these degradation products, or marker molecules, in the timbers of the Vasa and the Skuldelev ships. SEC also showed that the smaller the PEG size the higher the concentration in the wood. For the Vasa this means that PEG 600 is the main constituent of the PEG mixture in the wood. If the relative air humidity (RH) in a museum oscillates around $50\% \pm 5\%$, for example, then the water content of PEG 600 changes more than that of PEG 4000 as seen from sorption isotherms. It is of interest to have an object that responds as little as possible to climate fluctuations. In this respect the high PEG 600 content makes the Vasa more sensitive than the Skuldelev ships. There has been speculation that fluctuations in RH could be related to salt precipitations on the surface of the Vasa since the precipitations showed up during a period with highly unstable RH. The RH was high during the day and low during night. The idea is that hydrated PEG dissolves salts from the material at high RH and that the salts precipitate on the wood surface when the PEG is dehydrated at low RH. Most aspects of these thoughts remain un-investigated and speculative. SEC showed how the individual PEG types in the Vasa are distributed. The profiles can be explained if one PEG type washes out of the wood, when treatment with this is followed by treatment with another PEG type. This results in a distribution where the concentration of the first PEG types applied peaks a few centimetres below the wood surface, and the PEG type that was applied last has a constant concentration through the depth of the wood.

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Appendix 2

Article II:
Mortensen, M.N., Egsgaard, H., Hvilsted, S., Shashoua, Y., Glastrup, J., Stability of polyethylene glycol in conserved wooden shipwrecks – the effect of matrix. Intended for Journal of Archaeological Science.

Stability of polyethylene glycol in conserved wooden shipwrecks – the effect of matrix

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Keywords
Archaeological wood, polyethylene glycol, PEG, stability, matrix, antioxidant, Vasa, Skuldelev

Abstract
The degradation of the polyethylene glycol (PEG) model molecule tetraethylene glycol (TEG) was studied at 70 °C under dry air and nitrogen. Degradation products were detected using gas chromatography-mass spectrometry (GC-MS). They were mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol and formic acid. The rate of degradation was significantly decreased by low concentrations of KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2, $CuSO_4$, fresh oak wood sawdust and gypsum-containing scrapings from the Vasa. Thus some components of archaeological wood matrix are able to inhibit oxidative degradation of TEG. $NaFe_3(SO_4)_2(OH)_6$ (Natrojarosite), FeS_2 (pyrite), $FeSO_4$, $Fe_2(SO_4)_3$, $NiCl_2$, $NiSO_4$, Fe, Cu, Fe_2O_3, CuO, $NaHSO_4$ and natrojarosite-containing scrapings from the Vasa had no major effect on the rate of oxidation.

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Introduction

PEG (polyethylene glycol) impregnation is the method of choice for dimensionally stabilising waterlogged archaeological wooden objects today and it has been since the sixties (Morén and Centerwall, 1960; Seborg and Inverari, 1962). Thus, many waterlogged archaeological wooden shipwrecks that have received such treatment, are exhibited on museums around the world today. A few examples include the Swedish warship Vasa (Håfors, 1990; Håfors, 1999; Håfors, 2001), the Danish Skuldelev Viking ships (Jensen *et al*., 2002) the German Bremen cog (Clariant, 2000; Hoffmann *et al*., 2004) and the Batavia in Australia (Unger and Schniewind, 2001). The Mary Rose in Portsmouth England is currently being impregnated (Sandström *et al*., 2005). The Vasa and the Skuldelev timbers have been characterised in many different ways. In one study the molecular weights of PEG in the Vasa and the Skuldelev ships, were characterised. The molecular weights did not seem lower than expected, except in one case where a low molecular fraction of PEG was considered as a possible degradation product (Mortensen *et al*., 2007). In another study formic acid was found in the PEG treated timbers of many different shipwrecks including the Vasa and the Skuldelev ships and it was discussed whether this could be a product of PEG degradation (Glastrup *et al*., 2006). The Danish Hjortspring boat was re-impregnated in the sixties and surplus PEG was melted from the wood at 80°C. Here it was clearly shown that severe PEG degradation had taken place during this treatment (Padfield *et al*., 1990).

The stability of PEG and its degradation mechanism have been studied extensively in many different accelerated ageing experiments (Bilz *et al*., 1994; Costa *et al*., 1992; Decker and Marchal, 1970; Dulog, 1967; Geymayer *et al*., 1991; Glastrup, 1996; Glastrup, 2003; Goglev and Neiman, 1967; Han *et al*., 1995; Han *et al*., 1997; Lloyd, 1963; Madorsky and Straus, 1959; Mcgary, 1960; Mikhal'chuk *et al*., 2004; Mkhatresh and Heatley, 2004; Mkhatresh and Heatley, 2002; Scheirs *et al*., 1991; Yang *et al*., 1996). For example, one experiment suggests a mechanism where oxygen reacts with PEG to yield shortened PEG chains, formic acid and an unstable hemiacetal that breaks up to form the alcohol (shortened PEG) and formaldehyde (Glastrup, 1996). In a similar experiment it turned out that triethylene glycol molecules with modified termini degraded at different rates, this pointed at degradation at the terminal monomeric units exclusively (Glastrup, 2003). In contrast Heatly and co-workers have proposed that the PEG degradation is a random chain scission (RCS) (Mkhatresh and Heatley, 2004; Mkhatresh and Heatley, 2002; Yang *et al*., 1996). An in-chain hydroperoxide breaks up the PEG chain yielding a formate and a hemiacetal that again is split into an alcohol and formaldehyde. In both of these mechanisms esterification of formic acid and the alcohols (and hydrolysis of the esters) is considered as natural behaviour of the products formed.

Clearly PEG impregnated waterlogged archaeological wooden objects not only contain PEG. Wood in itself is a complex mixture of lignin, cellulose, hemicellulose and then there is the degradation products of all these components in the waterlogged archaeological wood. Furthermore the marine environment where the shipwreck has been before salvage also has an influence on the composition of the material of the object. One example that has been described is the uptake of sulphur compounds by the wood from the surrounding marine environment. Iron is another compound that is often buried along with wooden shipwrecks in the shape of nails or cannons. The iron is incorporated into the wood over time, often together with sulphur, such as for example in pyrite (FeS_2) (Crumlin-Pedersen and Olsen, 2002; MacLeod and Kenna, 1991; Sandström *et al*., 2002; Sandström *et al*., 2005).

It is thus clear that PEG in the matrix of wooden shipwrecks has a very large number of compounds that, at least in theory, might affect its degradation. There could be components that accelerate the degradation of PEG and there could also be components that slow it down. This effect could act through interference with the degradation reaction of PEG. Another scenario could be if matrix elements react more readily with oxygen than does PEG. This would protect PEG without interfering with its degradation mechanism. The present study attempts a look into some of these questions.

In the present work accelerated ageing of the PEG model molecule TEG (tetraethylene glycol) was carried out by monitoring the disappearance of TEG and by characterising the degradation products formed. The effect of 20 different compounds on the rate of degradation of TEG in this setup was investigated. Some of the compounds have been identified in PEG treated wooden shipwrecks, others have been investigated in different experiments in the literature. It turns out that some of the compounds actually affect the stability of TEG, even though they were present in catalytic amounts only.

Experimental

Setup

Accelerated ageing of TEG was carried out in screw cap glass vials (45 mm x 15 mm) with Teflon membranes in the lid and two 1.6 mm I.D. (1/16 inch) Teflon tubes inserted through the membrane. One tube led gas (air or nitrogen) into the vial, all the way down to the bottom of the vial so that the gas was bubbling through the liquid contents (TEG) of the vial. The other Teflon tube came out just under the membrane, in order to lead gas out of the vial without driving out the liquid contents too. The vial (referred to as the reaction vial) was situated in an oven at 70 °C, the exit tube led gas out of the reaction vial into a second vial (referred to as the condenser vial) in a -10 °C cryostatic bath. The condenser vial was built the same way as the reaction vial with two 1.6 mm I.D. Teflon tubes passing through the Teflon membrane. The inlet tube from the reaction vial in the oven reached down to the bottom of the condenser vial to get a large surface for condensing volatile reaction products formed in the reaction vial. The exit tube was also situated just under the membrane in the lid, in order to prevent the liquid contents from leaving through the tube. In this setup TEG and reaction products only got into contact with glass and Teflon, the entire setup was kept in the dark. 25 reaction vials, each connected to 25 condenser vials, were at disposal. A steady gas flow in all 25 vials was ensured by flow restrictors (narrow inserts 0.25 mm I.D.) in the Teflon tubes leading to the reaction vials. A high pressure built up before the restrictors which made the airflow in one tube almost independent of the resistance of the other 24 tubes even though they all fed from the same gas source. The air used was cleaned and dried by passing it through cartridges with activated coal and a cartridge containing 4 Å ($4*10^{-10}$ m) molecular sieves. The nitrogen gas used was 99.998 % pure. The flow of gas, air or nitrogen, was adjusted to 10 ml/min. Each reaction vial was charged with 2.00 ml TEG and in one series of measurements 10 mmol/l of an additive described later. In some cases it was necessary to dilute the samples further (1 part to 9) to optimise chromatography on the GC-MS (Gas Chromatography-Mass Spectrometry), this was done with pure acetone so that the concentration ratio between TEG and naphthalene was maintained. The samples were kept in the freezer at approximately –20 ºC until they were analyzed on the GC-MS.

Accelerated ageing
Two series of ageing of 99 % pure tetraethylene glycol (TEG), were carried out. In the first series four vials were run. They contained TEG under dry air (vial A), TEG under nitrogen (vial B), TEG with 0.011 mol/l potassium iodide under dry air (vial C) and TEG with 0.013 mol/l potassium iodide under nitrogen (vial D). The vials were weighed every five days at the least and 2.0 µl samples were taken out for GC-MS with a 10 µl Hamilton syringe. The 2.0 µl samples were dissolved in 1.000 ml acetone containing naphthalene (0.5045 g/l) as internal standard, in 2 ml GC-auto sampler vials. In this series GC-MS was used to quantify the TEG content over time and to identify reaction products.

In the second series of ageing experiments 20 different additives were screened for effect on TEG degradation under air. The vials were sampled for GC-MS analysis and weighed three times during 31 days of ageing. The concentration of additive in the 2.00 ml TEG in a reaction vial was aiming at 10 mmol/l, the exact mass added is given in parenthesis below. Many different qualities of chemicals were used, the following were pro analysi; Cu (1.33 mg), $Fe_2(SO_4)_3 \cdot 5H_2O$ (10.17 mg), $NiSO_4 \cdot 7H_2O$ (5.25 mg), $NiCl_2 \cdot 6H_2O$ (6.87 mg), $NaHSO_4 \cdot H_2O$ (7.64 mg), $FeCl_3 \cdot 6H_2O$ (6.44 mg), $Cu(CH_3COO)_2 \cdot H_2O$ (4.23 mg), $FeSO_4 \cdot 7H_2O$ (5.48 mg) and KI (3.99 mg). The $CuSO_4 \cdot 5H_2O$ (5.12 mg) used was re-crystalised from water before use. The MnO_2 (2.31 mg) was of synthesis quality (>90%). A few substances were collected in nature, these include $NaFe_3(SO_4)_2(OH)_6$ (natrojarosite) (9.86 mg) collected as a pure crystalline mineral in Mexico, FeS_2 (pyrite) (3.07 mg) was collected as the pure crystalline mineral in Norway and the fresh oak sawdust (14.19 mg), was made from a dried oak plank that had not been impregnated or chemically treated in any way. CuO (1.59 mg) was prepared by precipitating the hydroxide from an aqueous solution of $CuSO_4$ by addition of sodium hydroxide. The precipitate was isolated by centrifugation and washed with distilled water. The isolated hydroxide was then dehydrated in a porcelain crucible on a heating plate set to 200 °C for an hour, until all of the blue hydroxide was turned into black CuO powder. Fe_2O_3 (3.63 mg) was used as the natural pigment known as “burned Sienna” which was prepared in much the same way as the copper(II)oxide. Fe powder (1.44 mg) was made from iron nails ground on sandpaper. Two scrapings from the Vasa were used, one containing natrojarosite (24.25 mg) and the other containing gypsum (19.51 mg). The mineral contents of both samples were based on the evaluation of colour and appearance of the precipitates on the Vasa wood, done by conservator at the Vasa Museum, Emma Hocker. Besides the minerals, the scraping may also contain wood dust and PEG.

Instrumentation
The concentration of TEG, and presence of tri- and diethylene glycol with and without formates, in the reaction vials, were determined using a GC-MS equipped with a Varian 8200 autosampler, the GC was a Varian 3400 Cx and the MS a Varian saturn 4D. The column was an Agilent DB-1701, length (L) 8 m, I.D. 0.18 mm, inner coating film 0.4 µm. The carrier gas used was He 99.9995 % and a front column pressure of 10 psi (69 kPa). The autosampler was set to inject 0.2 µl sample. The temperature program for the injector was; hold 48 °C for 0.1 min; ramp 48 °C to 220 °C at 200 °/min; hold 220 °C for 4 min. The column temperature programme was; hold 48 °C for 0.5 min; ramp 48 °C to 220 °C at 15 °/min; hold 220 °C for 4 min. The transfer line was 220 °C and the trap temperature was 220 °C. The MS scanned from *m/z* 28 to 399, and averaged two scans every 0.5 s. The TEG concentration was calculated from TIC (Total Ion Count) areas of the TEG analyte relative to the TIC area of the naphthalene internal standard. Four standard solutions were run with every carousel of samples. They contained 0.500 µl TEG per ml acetone, 1.00 µl TEG per ml acetone, 1.50 µl TEG per ml acetone and 2.00 µl TEG per ml acetone respectively all with

0.5045 g/l naphthalene. The ratios of the TEG to naphthalene areas from these standards were averaged and used to calculate the amount of TEG in the samples in that carousel. The retention times were; naphthalene 6.04 min, TEG 10.04 min, tetraethylene glycol monoformate 10.75 min, tetraethylene glycol diformate 11.33 min, triethylene glycol 7.63 min, triethylene glycol monoformate 8.37 min, triethylene glycol diformate 9.05 min, diethylene glycol 5.07 min and diethylene glycol monoformate 5.58 min. The identities of the ethylene glycol peaks were confirmed by comparing retention time and mass spectra to recordings of the relevant reference chemicals. It was easy to recognise a peak corresponding to a formate because it was dominated by *m*/*z* 73 corresponding to the fragment ion $[CH_2CH_2OCOH]^{+\bullet}$. Distinction between chromatogram peaks representing monoformates and diformates of the same ethylene glycol was much more difficult because they have identical mass spectra (dominated by *m*/*z* 73). Here the assignment was based on the assumption that monoformates are formed in larger quantities than diformates during accelerated ageing of TEG and during esterification of TEG with formic acid, performed as reference experiment.

The condensates were analysed on a Varian 3400Cx GC with He 99.9995 % as carrier gas at a head pressure of 25 psi (172 kPa). A Varian 8200 autosampler injected 0.5 µl sample onto an on-column injector with the following temperature program; hold 50 °C for 0.1 min, ramp 50°C to 220°C at 200 °/min, hold 220 °C for 15.05 min. The column was a Restek Stabilwax-DA, L 30 m, I.D. 0.25 mm, coating 0.25 mm. The oven temperature program was; hold 50 °C for 0.5 min.; ramp 50 °C to 150 °C at 50 °/min; hold 150 °C for 5 min; ramp 150 °C to 220 °C at 20 °/min; hold 220 °C for 10 min. The transfer line to the MS was mounted with a No-Vent module and the temperature was 220 °C. The MS used was a Varian Saturn 2000 instrument with Silcosteel treated ion-trap electrodes (trap temperature 190 °C). The MS scanned from *m/z* 19 to *m/z* 249, and averaged two scans every 0.5 s with SIS (Selective Ion Storage) to allow filtering of *m/z* 28 and *m/z* 32. Retention times were; naphthalene 9.92 min, formic acid 5.88 min (which has a very characteristic trace of *m*/*z* 47, protonated molecules are readily seen on this instrument), monoethylene glycol 7.71 min, monoethylene glycol monoformate 5.97 min (overlaid with formic acid) monoethylene glycol diformate 7.78 min (overlaid with mono ethylene glycol). The retention time for diethylene glycol was 11.78 min, diethylene glycol monoformate 11.64 min and diethylene glycol diformate 11.56 min. On this GC-MS, discriminating mono- and diformate estes gave the same problems as described above, they were solved the same way too.

ATR FT-IR (Attenuated Total Reflectance Fourier Transform-Infrared) spectra were collected over 30 scans at a resolution of 4 cm^{-1} between 4000 cm^{-1} and 600 cm^{-1} (the lower limit of sensitivity for ATR) using an ASI DurasamplIR 1 single reflection accessory with an angle of incidence of 45° and fitted with a diamond internal reflection element in a Perkin–Elmer Spectrum 1000 FT-IR spectrometer. Background spectra of the empty, clean accessory open to air were run just before the samples. A drop of sample liquid was placed on the diamond, the torque screw was tightened over the liquid and the spectra were collected.

Results and discussion

Figure 1 **A** and **B** shows the results of accelerated ageing of tetraethylene glycol (TEG). The number of moles TEG that is in the reaction vial, determined by GC-MS, is plotted versus days of ageing at 70 °C.

When dried air is passed through the vial (figure 1 **A**), the TEG content is initially around 30 mmol. Over the first 2-3 days of ageing there seems to be a lag phase where the TEG content remains constant. This could due to the build up of reactive degradation initiators prior to TEG consumption. After the lag, TEG is consumed from the vial and after about 20 days there is less than 10 mmol TEG in the vial meaning that basically all the TEG that was there initially has reacted.

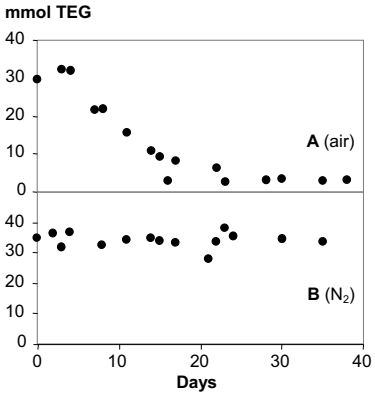


Figure 1. Total amount of TEG (mmol) in a reaction vial plotted versus days of ageing. The vials contain pure TEG, one was aged under dry air (A) the other under nitrogen (B). TEG is degraded in air in about 18 days under nitrogen it is not degraded.

When the experiment is performed with nitrogen passing through the vial instead of air, the TEG content remains constant around 35 mmol for the entire 35 days of ageing (figure 1. **B**). Thus, no degradation takes place under nitrogen. This demonstrates that the reaction that consumes TEG in the first experiment (figure 1. **A**) is an oxidation.

An experiment like the one in figure 1. was conducted with catalytic amounts (approximately 10 mmol/l) of potassium iodide dissolved in the TEG. The results are shown in figure 2 where it is seen that the TEG contents are constant around 35 mmol throughout the duration of both the experiment with air passing through (figure 2. C) and the one with nitrogen passing through (figure 2. D). Thus when potassium iodide is added TEG is not degraded. The TEG is just as protected when air is passed through the vial with potassium iodide as when nitrogen is passed through.

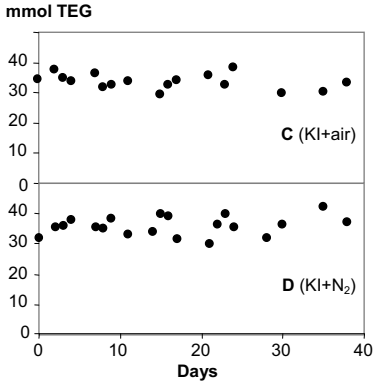


Figure 2. Total amount of TEG (mmol) in a reaction vial plotted versus days of ageing. The vials contain TEG and ca. 10 mmol/l potassium iodide, one was aged under dry air (C) the other under nitrogen (D). No degradation takes place in any of these vials.

This means that potassium iodide prevents degradation of TEG at 70 °C under dry air.

The ageing experiments described in figure 1. **A, B** and figure 2. **C** and **D**, were allowed to continue. The results are shown in figure 3. where the weight of the contents of the reaction vials in percent of the initial weight of the contents of the reaction vials, is plotted versus days of ageing. When comparing the overall shape of the weight-loss curves in figure 3 with the corresponding moles of TEG in figure 1 and 2 it is seen that they look similar. One difference is that the degradation appears faster when the number of moles is considered. This is because both volatile reaction products and remaining TEG contribute to the weight of the vials. If this is kept in mind, the weight-loss can be used as a measure of TEG degradation.

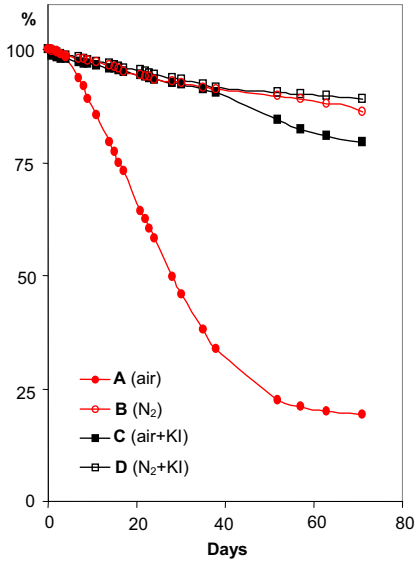


Figure 3. The mass of the reaction vials in percent of their start mass is plotted versus days of ageing. Circles: pure TEG (closed: air (A), open: nitrogen (B)). Squares: TEG with ca. 10 mmol/l potassium iodide (closed: air (C), open: nitrogen (D)). Pure TEG loose weight under air, TEG with potassium iodide does not. Under nitrogen very little weight-loss is observed.

The degradation observed for aerated TEG in figure 3 **A** is almost complete after 50-60 days. At this point the vials contained a thick sticky substance that cannot be sampled with a Hamilton syringe for GC-MS nor can air be bubbled through it. The other three curves in figure 3., **B, C** and **D** lose little weight over 71 days. This moderate loss is ascribed to either evaporation of TEG or a little pyrolysis, or both, because oxidation is not an option in the TEG under nitrogen (**B**). The curves for TEG with potassium iodide under air (**C**) look a lot like TEG under nitrogen (**B**) over 71 days. This demonstrates a very persistent antioxidant effect of the 10 mmol/l potassium iodide added. Even after 518 days (data not shown) there is an effect, the vial with potassium iodide and air (**C**) is on the same level as the vial with TEG and nitrogen (**B**) or with TEG, KI and nitrogen (around 20 %). This is higher than for the aerated TEG (**A**), which is on 5.5 % after 518 days. Two vials containing TEG and approximately 10 mmol/l of iron(III)sulfate were run, the iron(III)sulfate

had no effect on the degradation of TEG under air (degraded) or nitrogen (did not degrade) over 518 days (data not shown).

A range of different compounds were screened for effect on TEG stability, these are shown in table 1. They were tested in the setup described, at 70 °C with air passing through the vials containing TEG and the additive in catalytic amounts (exact values in the experimental section). All of the vials were weighed three times in total over the 31 days that this experiment lasted. The content of the vial containing TEG without any additives went from weighing 2.00 g on day one to 0.75 g (38 % of the initial weight) on day 31. Thus the setup is clearly sensitive to the kind of change in question for degradation. The slope of a straight line through the three points measured gave a rate of weight-loss of 0.04 g/day for the TEG control vial (table 1.).

A large part of the additives tested had a slope similar to that of the TEG control (0.04±0.01 g/day) and thus no major influence on the rate of TEG degradation (table 1.). Some of these include iron powder (Fe), iron oxide (Fe_2O_3), pyrite (FeS_2), iron(II)sulfate ($FeSO_4 \cdot 7H_2O$), iron(III)sulfate ($Fe_2(SO_4)_3 \cdot 5H_2O$), natrojarosite ($NaFe_3(SO_4)_2(OH)_6$) and a scraping from the Vasa containing natrojarosite precipitation. These compounds are all related to groups of minerals detected on waterlogged wooden objects. For example on the Batavia the following species were identified αFeO(OH), FeO(OH), $Fe(OH)_2$, S, FeS_2, $FeSO_4 \cdot 4H_2O$, $FeSO_4 \cdot 5H_2O$, $NaFe_3(SO_4)_2(OH)_6$, $KFe_3(SO_4)_2(OH)_6$, $Fe_3(SO_4)_4 \cdot 22H_2O$, $FeSO_4(OH) \cdot 2H_2O$ and $Fe_3(SO_4)_4 \cdot 14H_2O$ (MacLeod and Kenna, 1991). On the Mary Rose FeS_2, Fe_8S_9, $FeSO_4 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$ and $NaFe_3(SO_4)_2(OH)_6$ was identified (Sandström *et al*., 2005). In the Vasa $CaSO_4 \cdot 2H_2O$, $NaFe_3(SO_4)_2(OH)_6$, $FeSO_4 \cdot 7H_2O$ and S_8 was identified (Sandström *et al*., 2002; Sandström *et al*., 2003) and on the Skuldelev ships around the nail holes $FeSO_4 \cdot 4H_2O$ was identified (Crumlin-Pedersen and Olsen, 2002). All these substances were detected using various X-ray techniques. All of these iron salts probably originate from the free iron in the nails or from cannonballs buried with the ship and therefore Fe powder was included in the tests which had no effect either. The presence of these salts in and on the wood may have mechanical effect on the wood tissue but they do not seem to have any chemical effect on tetraethylene glycol in the current experiment. It is clear that the hydration of the salts, for example $FeSO_4 \cdot XH_2O$, is not controlled in this experiment because the air or nitrogen that is passed through the vials is dried and thus dries out whatever is in the vials, probably also crystal water. It does however give a good indication of activities of the ions themselves. Also the scrapings from the Vasa containing natrojarosite did not affect TEG in this experiment.

Some of the components in the group without any effect on TEG degradation (table 1.), have been tested by others too. These include $FeSO_4 \cdot 7H_2O$, $Fe_2(SO_4)_3 \cdot 5H_2O$, $NiSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$ (Glastrup and Padfield, 1993; Lloyd, 1963; Mcgary, 1960). Iron(III)sulfate and nickel(II)chloride for example were found to be either completely inactive (iron(III)sulfate) or only slightly active (nickel(II)chloride) as PEG stabilisers, by Lloyd (1963). The low activity of nickel(II)chloride found by Lloyd is well within the uncertainty of the present experiment, thus there is agreement between this study and others.

Table 1. Components tested for effect on TEG stability.

Group	***Additive***	***Rate (g/day)***
TEG	None	0.04
Degraded at same rate as TEG	*$NaFe_3(SO_4)_2(OH)_6$ (Natrojarosite)*	*0.04**
	Scraping from the Vasa containing Natrojarosite	*0.05**
	FeS_2 (pyrite)	*0.05**
	$FeSO_4 \cdot 7H_2O$	***0.05****
	$Fe_2(SO_4)_3 \cdot 5H_2O$	***0.05****
	$NiCl_2 \cdot 6H_2O$	**0.04***
	$NiSO_4 \cdot 7H_2O$	**0.05**
	Fe	0.04*
	Cu	0.03*
	Fe_2O_3	0.05*
	CuO	0.04*
	$NaHSO_4 \cdot H_2O$	0.04*
Degraded slower than TEG	*Fresh oak sawdust*	*0.02**
	Scraping from the Vasa containing gypsum	*0.01**
	KI	**0.002**
	$FeCl_3 \cdot 6H_2O$	**0.003**
	$Cu(CH_3COO)_2 \cdot H_2O$	**0.003**
	MnO_2	0.003*
	$CuSO_4 \cdot 5H_2O$	0.004

Bold: Substance described in the literature in relation to PEG stability (Costa *et al*., 1992; Glastrup and Padfield, 1993; Lloyd, 1963; Mcgary, 1960).
Italic: Substance related to compounds detected in archaeological wood (Crumlin-Pedersen and Olsen, 2002; MacLeod and Kenna, 1991; Sandström *et al*., 2002; Sandström *et al*., 2005).
*Additive not fully dissolved in the TEG

Some compounds affected the rate of TEG degradation significantly. Among the tested components were fresh oak sawdust, scrapings from the Vasa containing gypsum, copper(II)sulfate, iron(III)chloride, copper(II)acetate, manganesedioxide and potassium iodide. These components all reduced the rate of weight-loss of TEG by at least a factor of two (rate ≤ 0.02 g/day), thus they protected the TEG from oxidation. Potassium iodide was a good inhibitor of PEG thermooxidation, in the experiments in figure 2 and in the experiments done by Costa *et al*. (1992). Costa *et al*. described potassium iodide along with other iodides in a thermogravimetric experiment under air at 322 °C. Lloyd *et al*. (1963) reported that iron(III)chloride was also a good antioxidant in an experiment with diethylene glycol at 75 °C under 1 atm (0.1 Mpa) oxygen, and in some cases addition of an azo initiator (2,2´-azobis(2-methylpropionitrile)). This is all in agreement with the observations made here.

Some of these compounds, including MnO_2, $CuSO_4$, and $Cu(CH_3COO)_2$, inhibited TEG oxidation even though they were not fully soluble in the TEG. This suggests that the compounds can act as heterogeneous antioxidants. Thus the effect of an additive is not given by its solubility alone (nickel sulfate is soluble and ineffective, manganese(IV)oxide is insoluble and effective), neither is it given by the cation or the anion alone. For example iron(III) is found both in effective and ineffective salts ($FeCl_3$ effective and $Fe_2(SO_4)_3$ ineffective). The same can be said for the sulfate ion and the chloride ion.

It is interesting to note that oak wood sawdust retards thermooxidation of TEG (table 1.) although not as effectively as some of the salts. The literature supports this observation, Mikhal´chuk *et al*. (2004) found that several plant phenols (herb extracts containing caffeic acid, syringic acid and phloroglucinol) inhibit thermooxidation of PEG at 80 °C. Other experiments confirm that wood contains components with antioxidant properties (Ebringerov *et al*., 2008; Kosikova *et al*., 2006; Zulaica-Villagomez *et al*., 2005). Han *et al*. (1997) found that polyphenole antioxidants, similar to lignin in structure, protect PEG 6000 from oxidation at 80 °C.

In the group of components that had an antioxidant effect in this experiment two are definitely present in the Vasa matrix, namely wood and the scraping from the Vasa containing gypsum. Some of the other compounds that were effective antioxidants may or may not be in the Vasa or in other waterlogged wooden objects, this is not known. Whether the effect of the scraping was due to gypsum or perhaps to wood dust in the sample is hard to say but it is interesting to realize that some components of the Vasa, retard degradation of TEG.

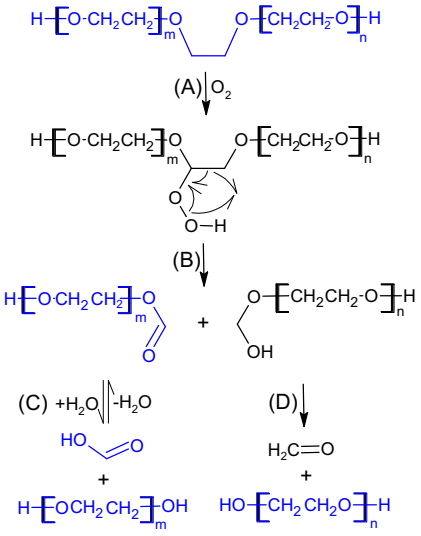
The degradation products formed during ageing of pure TEG and of TEG with additives, were identified using GC-MS and ATR FT-IR. It seems that the species formed during ageing of TEG with and without additives are the same, the only difference observed relates to the rates of formation. In the reaction vials the TEG content decreased as the mono- and diformate of TEG increased. It was also seen that the tri- and diethylene glycol content increased as the content of mono- and diformates of tri- and diethylene glycol increased. The condensates contained diethylene glycol and diethylene glycol mono- and diformate, monoethylene glycol, monoethylene glycol monoformate, monoethylene glycol diformate and formic acid. It seems that the more volatile compounds predominate in condensates. ATR FT-IR spectra (not shown) of the condensates had intense carbonyl peaks at 1709 cm^{-1} and the pH in the condensates were as low as 1-2 (pH of pure TEG is 5). This is in agreement with the GC-MS result that formic acid and formates are in the condensates. The finding of these reaction products is not new in itself, the products and the order of appearance of the products over time of ageing, has been identified previously (Glastrup, 1996). Thus it can be confirmed here that TEG oxidation leads to mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol and formic acid.

Further discussion

Other information was obtained besides the identity of the reaction products. The amount of additive was small compared to the amount of TEG (ca. 580 TEG molecules per additive molecule). This information in combination with the very persistent antioxidant effect, suggests a catalytic mechanism for the antioxidant. The small amount of antioxidant would have been used up far earlier than 518 days, if there had been a stoichiometric reaction between antioxidant and oxygen in the air. Thus the mechanism should involve an element of catalysis (no exhaustion of antioxidant) furthermore many different salts should be able to catalyze the same reaction. Hydroperoxides of PEG have been identified in the breakdown of PEG (Goglev and Neiman, 1967; Mkhatresh and Heatley, 2004), thus removal of hydroperoxides could inhibit degradation. It could be speculated that this takes place analogously to the catalytic disproportionation of hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O + O_2$). This reaction is catalyzed by many different compounds (alkali, heavy metal ions, heterogeneous catalysts like MnO_2 and Pt) (Housecroft and Sharpe, 2001) in analogy to the observations made here.

A mechanism was discussed by Lloyd (1963) where metal ion-hydroperoxide coordination mediated hydroperoxide breakdown catalytically. It seems plausible that the additives with antioxidant effect investigated here, could act by breaking down the TEG-hydroperoxides catalytically.

It is normal to have a number of processes involving the radicals; alkyl ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$) and involving hydroperoxides (ROOH) in a room temperature reaction between oxygen and e.g. a polymer. However, it is possible to explain the products detected in the present thermo-oxidation of TEG by the mechanism proposed in scheme 1. It involves an initial hydroperoxide intermediate, it consumes TEG, it produces mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol and formic acid in accordance with the observations. The species in blue (formates, formic acid and oligoethylene glycols) were detected by GC-MS.



Scheme 1. Proposed degradation mechanism for tetraethylene glycol (for TEG m+n+1=4). A hydroperoxide is formed on TEG in reaction A, for an in-chain hydroperoxide both m≠0 and n≠0. Rearrangement of the hydroperoxide (reaction B) leads to a formate and a hemiacetal. The formate is in equilibrium with the alcohol and formic acid (reaction C) via esterification/hydrolysis. The unstable hemiacetal breaks down to formaldehyde and the alcohol in reaction D. A hydroperoxide situated at a terminal monomeric unit would produce either formic acid (m=0) or methanediol (n=0) depending on the position relative to the hydroxo group. Methanediol would dehydrate to give formaldehyde (reaction D, n=0). The species in blue (formates, formic acid and oligoethylene glycols) were detected by GC-MS.

In the mechanism tetraethylene glycol (m+n+1=4) is degraded but the mechanism should be valid for larger PEG molecules too. In the first step it is suggested that a hydroperoxide is formed (reaction A Scheme 1.) on the TEG (or the polymer). If this is situated in-chain (both m≠0 and n≠0) the rearrangement shown in reaction B Scheme 1. leads to a formate and a hemiacetal. The hemiacetal is unstable and it will rearrange to give formaldehyde and an alcohol (Scheme 1.,

reaction D). The formate is in equilibrium with the alcohol and formic acid (Scheme 1., reaction C) via esterification/hydrolysis. For a terminal hydroperoxide either formic acid (m=0) or methanediol (n=0) would be produced depending on the position in the terminal monomeric unit of the hydroperoxide. If methanediol is formed it will dehydrate fast under the conditions of the experiment (dry air at 70 °C), leading to formaldehyde and water (Scheme 1., reaction D, n=0). It is understood that the triethylene glycol and triethylene glycol mono formate produced from TEG in reaction B, -C and -D can enter the degradation process again in reaction A. The formaldehyde produced in reaction D was not observed in the GC-MS in this experiment. It is believed that the formaldehyde either evaporates out of the setup (bp –19 °C) or condenses to trioxane (bp 114 °C) that is oxidized to formic acid.

Heatly and co-workers have considered the breakdown of PEG by Random Chain Scission (RCS) in experiments with high molecular weight PEG (Mkhatresh and Heatley, 2004; Mkhatresh and Heatley, 2002; Yang *et al*., 1996). The reaction suggested is basically an RCS version of the reaction in scheme 1. Glastrup has suggested that the reaction may be an end degradation in an experiment with triethylene glycol where the end groups had been modified (Glastrup, 2003). The functional groups involved in this mechanism (Glastrup, 1996) are also accounted for in the mechanism given here (n=0 in scheme 1.). C-H bond homolysis is likely to proceed the installation of dioxygen between the C and the H to form the hydroperoxide, thus the relative stabilities of the radicals generated on the polymer chain are likely to determine the possible regioselectivity of the hydroperoxide in PEG. When comparing the possibility for radical hyperconjugation on an in-chain carbon with a carbon next to an alcohol in the end groups, the difference seems rather small. In both cases the hydrogen is bound to a carbon that has a methyl group and an oxygen atom as the nearest neighbours. If one possibility should be energetically favoured over the other it should be the in-chain radical because it has a long polymer chain on both sides of the radical to stabilize it, the terminal radical only has polymer chain on one side. This points to a random chain scission (RCS) rather than an end-degradation (m=0 or n =0 in scheme 1.). Clearly molecular weight has a lot to say in the matter. If the C-H bond energies were equal in the entire polymer, the probability for in-chain degradation in tetraethylene glycol would be 0.5 ((DP-end groups)/DP) but 0.98 for PEG 4000 (DP=91). In other words if the end degradation and in-chain degradation should take place an equal number of times in PEG 4000, the reactivity would have to be 49 times higher for the end groups than for in-chain groups (0.98/0.02). Although the mechanism, suggested in scheme 1., both accounts for products formed by end- degradation and by in-chain reactions it seems likely that large PEG molecules are more likely to take the in-chain route.

Perspectives to PEG treated wooden shipwrecks

Some of the products of TEG degradation established in the present work have been detected in PEG treated archaeological wooden objects. Formic acid has been detected in the Vasa, the Skuldelev ships, the Oberländer boat and the Bremen cog although in low concentrations (average 0.032 % by weight) (Glastrup *et al*., 2006). It still remains to be proven if this formic acid is a product of degradation of the PEG in these ships or a naturally occurring component in the concerned material. What could look like shortened PEG chains has been found in wood samples from the Vasa and the Skuldelev Viking ships (Mortensen *et al*., 2007). In this case it remains to be shown if the shortening has taken place after impregnation of the wood with PEG or before impregnation. These projects are ongoing.

Clearly the finding that scrapings from the Vasa and wood powder influence the stability of TEG not only relates to the Vasa but to all PEG impregnated waterlogged wooden objects because they all contain wood. The reaction conditions in the accelerated ageing of TEG at 70 °C with a steady flow of air into the liquid medium, is far from the situation in PEG impregnated waterlogged archaeological wood at room temperature such as for example the Vasa wood. The air is supplied more slowly to the PEG in the solid medium and the antioxidant properties of the wood in a museum object are not necessarily available to the PEG in the same way as in this experiment, the difference in temperature is clearly an important difference too. Nevertheless, all other things equal, wood protects PEG from thermo-oxidative degradation.

The salts that were found to have antioxidant effect such as KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2 and $CuSO_4$ have not been detected in PEG treated wooden shipwrecks, but it would not be surprising to find for example $FeCl_3$ because iron is present as seen from the pyrite, and chloride is present in seawater. Whether this is the case or not, trying to impregnate such objects with an antioxidant salt could be tempting. It could also be an advantage in the process of impregnating new waterlogged wooden finds with PEG, if antioxidants were present in the PEG solutions. This would allow the temperature of the PEG solution to be raised without damaging the PEG and the rate of diffusion of PEG into the waterlogged object would increase significantly. Care should be taken though since these compounds very well could have other effects on waterlogged wood, than just the antioxidant effect. Adding a large amount of modern wood with a large surface area to a PEG solution during hot treatment of archaeological wood, could be an interesting way of testing wood as a natural antioxidant for conservation.

Conclusions

Tetraethylene glycol is oxidized by dry air at 70 °C, this leads to the production of mono-, di- and triethylene glycol, mono- and diformates of mono-, di-, tri- and tetraethylene glycol and formic acid.

KI, $FeCl_3$, $Cu(CH_3COO)_2$, MnO_2, $CuSO_4$, fresh oak wood sawdust and scraping from the Vasa containing gypsum inhibits PEG degradation under these conditions. Since wood must be present in all wooden shipwrecks, the PEG-impregnation could be protected by the wood matrix.

$NaFe_3(SO_4)_2(OH)_6$ (Natrojarosite), FeS_2 (pyrite), $FeSO_4$, $Fe_2(SO_4)_3$, $NiCl_2$, $NiSO_4$, Fe, Cu, Fe_2O_3, CuO, $NaHSO_4$ and scraping from the Vasa containing natrojarosite had no major effect on the rate of oxidation. Some of these compounds are relevant to archaeological wood.

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Appendix 3

Glastrup, J., Shashoua, Y., Egsgaard, H., Mortensen, M.N., Formic and acetic acids in archaeological wood. A comparison between the Vasa Warship, the Bremen Cog, the Oberländer Boat and the Danish Viking Ships, Holzforschung 60 (2006) 259-264.

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Formic and acetic acids in archaeological wood. A comparison between the Vasa Warship, the Bremen Cog, the Oberländer Boat and the Danish Viking Ships

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Abstract

A method has been developed to analyse and quantify formic and acetic acids in archaeological and fresh wood. The method takes advantage of the fact that, in equilibrium, the gas-phase concentrations of formic and acetic acids over pulverised archaeological wood in a sulfuric acid solution are proportional to their absolute concentrations in wood. The method is based on automated solid-phase micro extraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS). Good linearity of the calibration curves was observed. The content of formic and acetic acids in archaeological wood from ships conserved with polyethylene glycol (PEG) was determined. The content of formic acid is related to the intensity of conservation and, hence, the PEG content in wood. Accordingly, formic acid found in the hull of the ships may partly be a result of PEG degradation. The formic acid content in the Vasa warship is, on average, not higher than in the other ships analysed. In contrast, the acetic acid content in PEG-preserved archaeological wood is lower than in fresh wood. The acetic acid content is age-dependent and is lowest in 1000-year-old wood. The acetic acid probably originates from the wood.

Keywords: acetic acid; analysis; archaeological wood; formic acid; SPME; gas chromatography; mass spectrometry.

Introduction

Polyethylene glycol (PEG) is by far the most commonly used material in the conservation of archaeological wood that needs to be dimensionally stabilised. The first applications were in the 1950s (Moren and Centerwall 1960; Christensen 1970) and in a short time PEG was generally accepted as the best material for treatment of archaeological wood. One of the major efforts in this regard was in 1961, when the Swedish warship Vasa was salvaged from the Stockholm archipelago, and it was decided to apply PEG to the entire hull (Håfors 1990, 1998). Treatment and reconstruction of the ship was finished in 1990 and a museum dedicated to the ship and its history was opened.

In 2001 it was discovered that areas on the hull were being attacked, probably by sulfuric acid. The acid originates from oxidation of sulfur encrusted in the hull during the years on the sea-bed in the Stockholm archipelago (Sandström et al. 2002, 2003; Fors 2005). It was decided to consign the preservation problem of the ship to an expert group, who started work in autumn 2003. In addition, high amounts of formic acid were found in samples from the hull (Edenbrink 2004; Westermark et al. 2005), and the question arose as to whether this could be a result of solvolysis of the wood because of the combination of wood and PEG together with sulfuric acid. This combination is known to give rise to levulinic and formic acids in surprisingly high yields at elevated temperatures (Bouchard et al. 1990, 2003; Vidal et al. 1992; Rezzoug and Capart 1996, 2002). Another possibility is the direct formation of formic acid from PEG (Glastrup 1996, 2003).

It is therefore important to determine the content of formic and acetic acids in archaeological wood that has been conserved with PEG, and to compare the results with those for non-conserved archaeological wood. For this purpose we use solid-phase micro extraction (SPME) for the analysis of formic and acetic acids in archaeological wood from old PEG-treated ships.

Materials and methods

Preparation and analysis of wooden samples

Only oak wood was analysed. Quantification of formic and acetic acids in archaeological and fresh wood was based on calibration curves prepared from the acids added to untreated archaeological and fresh wood, respectively. The wood was pulverised in a Culatti MFC-type hammer mill. Pulverised fresh wood was left in open air for 4 weeks to allow the evaporation of as much acetic acid as possible before using it for the preparation of standards. Archaeological wood was used directly. Standards were prepared by adding approximately 1.0% ($w\,w^{-1}$) of formic and acetic acids to dried and pulverised fresh or archaeological oak wood. The sample was thoroughly mixed. After at least 48 h of equilibration time, the wood powder was then diluted with homogenised wood of the same origin to concentrations of approximately 0.04, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8% by weight using thorough mixing. Prepared standards are stable for at least 6 months. Analyses of standards and samples were performed by weighing 10 ± 0.05 mg on a balance into a 2-ml autosampler vial. The vial was charged with 0.500 ml of

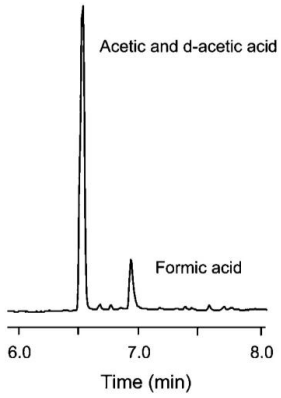

Figure 1 Chromatogram of formic and acetic acid analysed in the 1% standard.

1 M H_2SO_4, containing 1.00 ml l^{-1} of internal standard (CD_3COOD) using a 1-ml Gilson Pipetman. When analysing standards or samples from fresh wood, care was taken to analyse these after 24±1 h. When analysing standards or samples from archaeological wood, samples were analysed in a time interval from 24 to 33 h. Wooden samples with a waxy surface taken directly from archaeological wood were scraped or chipped into small pieces, to a size comparable to wood prepared using the hammer mill.

For SPME, the vials were head-space-extracted for 30 min using an SPME needle (Supelco, 57334-U, 85 μm Carboxen/PDMS Stableflex) mounted in a Varian 8200Cx autosampler, which automatically extracts components from the sample and transfers the needle to the injector block of the GC. The GC parameters were as follows: GC, Varian 3400Cx; carrier gas, He 99.9995%; head pressure, 1.7 bar (25 psi); injector, on-column SPI, temperature, initial 130°C for 0.1 min, ramp to 250°C at 300°C min^{-1}, hold for 3 min; analytical column, Restek Stabilwax-DA, 30 m, 0.25 mm i.d., coating 0.25 μm; oven temperature, initial 60°C for 2.5 min, ramp to 230°C at 30°C min^{-1}, hold for 3 min. The total analysis time was 11.17 min. The transfer line to the MS was mounted with an SGE No-Vent module at a temperature of 230°C. The MS used was a Saturn 2000 instrument with Silcosteel® treated ion-trap electrodes (190°C).

The MS scanned from *m/z* 19 to 249, and averaging of two scans every 0.5 s with SIS to allow filtering of *m/z* 28 and 32. The prescan target TIC for the ion trap was 5000.

MS data were collected for deuterated acetic acid at *m/z* 46, acetic acid at *m/z* 43, 60 and 61, and formic acid at *m/z* 29, 46 and 47. The instrument was autotuned. All calibration and analysis data were calculated as the ratio of peak areas for analyte/internal standard.

Results and discussion

Chromatographic performance

A chromatogram of the analysis of a 1% standard sample of formic and acetic acids is shown in Figure 1. The peaks show some tailing; however, this did not impair the quantification.

Calibration curves from fresh wood The formic acid calibration curve is presented in Figure 2a. Below a certain concentration, formic acid is bound to the wood structure to an extent that it is not released in 24 h. This results in a calibration curve with a negative intersection with *y* at $x=0$. The acetic acid calibration curve, Figure 2b, shows the presence of acetic acid in the recent oak wood. The positive concentration found at $x=0$, even if

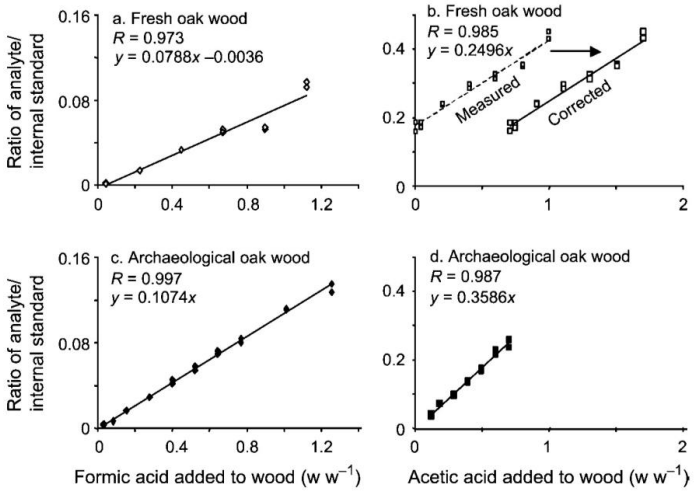

Figure 2 Calibration curves for formic (◇♦) and acetic (□■) acids in (a,b) fresh and (c,d) archaeological wood. Data are presented as the ratio of peak areas of the acids to the internal standard, as observed in the headspace above 10-mg standard samples after addition of 0.500 ml of H_2SO_4 solution, plotted against the concentration (w w^{-1}) in the standards. (b) Fresh wood used for calibration contained approximately 0.7% acetic acid. The calibration curve was corrected for this and the calibration range is therefore 0.71–1.71%. The standard archaeological wood contained formic and acetic acids, for which both curves were corrected as in b. The calibration range is (c) 0.034–1.256% for formic acid and (d) 0.077–0.702% for acetic acid.

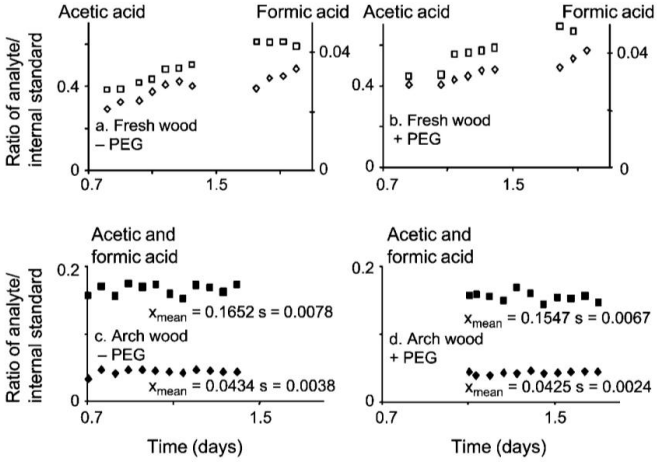

Figure 3 Amounts of formic (◇ ♦) and acetic (□■) acid released over time on addition of 0.5 ml of 1 M H_2SO_4 to 10 mg of a fresh (a,b) or archaeological (c,d) standard wood sample that was mixed with 0.4% of the acids in question.

no acetic acid was added to the wood, has to be taken into consideration and the graph has to be corrected so that it goes through zero (Figure 2b).

Calibration curves from archaeological wood Figure 2c,d shows the corresponding calibration curves for formic and acetic acids using approximately 1000-year-old archaeological wood from the Danish Viking Ships. This wood was dried without conservation, and thus preservation agents are not present.

The calibration curves for formic and acetic acids prepared on archaeological wood both showed a positive acid content and the calibration curves presented are corrected as illustrated in Figure 2b. The slopes of the calibration curves are greater than those for the calibration curves of recent wood.

The signal/noise (s/n) ratio was >10 for all positive samples analysed, except for the two formic acid analyses from dry wood obtained from the Danish Viking ships.

Sorption of formic and acetic acids in wood

The slopes of the calibration curves were lower when based on fresh wood compared to archaeological wood. This could be caused by only partial release of the acids from the fresh wood. We examined the desorption of formic and acetic acids from fresh wood with approximately 0.4% of formic and acetic acids added, after addition of 1 M H_2SO_4. The results in Figure 3a demonstrate that the measured amount of both formic and acetic acids increases over time. The experiment was repeated with the addition of 15% PEG 4000 (Figure 3b), revealing that the amounts of formic and acetic acids liberated are higher than in the absence of PEG. Therefore, not all the formic and acetic acids are liberated from fresh wood after the addition of the 0.5 ml of 1 M H_2SO_4 after 2 days. When analysing fresh wood, we therefore chose to analyse all samples after 24 ± 1 h. The figure also shows that the presence of PEG 4000 increases desorption from fresh wood and thus leads to erroneous results.

Figure 3c,d demonstrates that all formic and acetic acids were fully released from archaeological wood before 0.7 days. Student's t-test (unpaired, same variance) revealed that the average relative counts for both formic and acetic acids, with or without PEG, did not increase within the 95% interval. Quantification using archaeological wood as a calibration medium may therefore be used without correction for liberation of acid over time or for PEG content.

Discussion of calibration

The method presented represents a simplification of the determination of formic and acetic acids in wood compared to previous methods, which use simple extraction or basic hydrolysis and subsequent acidification of the acid distillate (Packman 1960; Arni et al. 1965; Balaban and Ucar 2003) and subsequent titration, GC (Bethge and Lindström 1974) or quantitative HPLC (Balaban and Ucar 2003).

Analysis of archaeological wood is straightforward. It has been argued (Balaban and Ucar 2003) that there is a possibility of the formation of formic acid using acid hydrolysis, but the constant amount of formic acid in the standards from 0.7 to 1.4 days shows that this is not the case here.

In fresh wood the conditions are different, and over time the standards containing formic and acetic acids release increasing amounts of acid, as observed in Figure 3a,b. For acetic acid, this could be due to gradual hydrolysis of acetyl groups bonded to the hemicelluloses (Balaban and Ucar 2003). The slope of the calibration curve from fresh wood is lower than that for archaeological wood (see Figure 2). This is because of the strong sorption of formic and acetic acids to fresh wood. Prolonged treatment of fresh wood in H_2SO_4 solution might lead to full desorption of the acids, which in turn would equili-

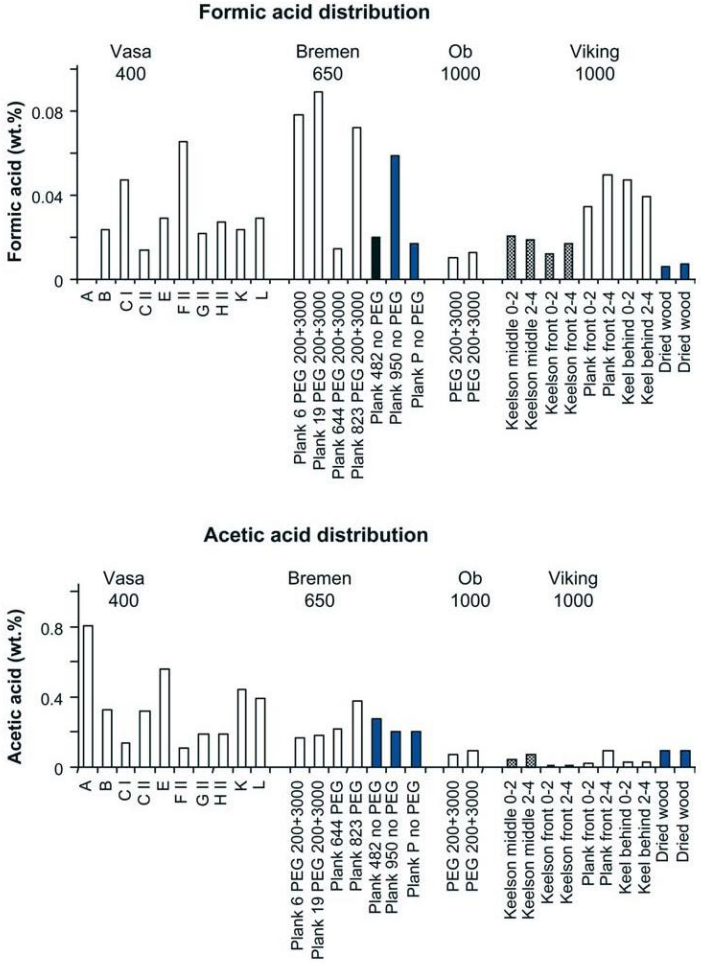

Figure 4 Concentrations of formic and acetic acids found in samples from the Vasa, the Bremen Cog, the Oberländer Boat and the Danish Viking Ships. The approximate ages of the ships are shown above the bars. Bars in black are samples not impregnated with PEG. From the Danish Viking Ships, the first four samples are from unusually sound PEG-impregnated wood (dotted bars), and the next four are from wood in poor condition, probably with a high PEG concentration.

brate with the gas phase, leading to easier quantification of the formic and acetic acids content. This has not been tested.

Content of formic and acetic acid in archaeological and fresh oak wood

Samples from four archaeological shipwrecks are shown in Figure 4, the Vasa, which is approximately 400 years old, the Bremen Cog, which is approximately 650 years old, the Oberländer Boat and the Danish Viking Ships, which are approximately 1000 years old. All four ships have been treated with PEG. A total of 31 samples were analysed, of which two were recent and 29 were archaeological wood. The wooden samples from the Vasa were – most of the time – treated by spraying with PEG dissolved in water. This process lasted for 17 years. Wood analysed from the other three shipwrecks, the Bremen Cog, the Oberländer Boat and the Danish Viking Ships, was either dried directly (five samples) or treated by submersion in PEG dissolved in water.

The formic acid content in all samples varied from below the detection limit in fresh wood to a maximum of 0.089% in archaeological wood, with an average (Av.) of 0.032%. Our findings are in contrast to previous results (Westermark et al. 2005) showing that four analyses of formic acid in the Vasa hull yielded concentrations between 0.2% and 1.0% in wood. Our results are approximately a factor of 10 lower.

In dried samples from the Danish Viking Ships and the Bremen Cog, the average formic acid content was 0.022% in dried wood and 0.035% in PEG-treated wood. In PEG treated samples from the Danish Viking Ships, four samples were from wood in good condition, whereas the other four were from wood in poor/softer condition. The good wood samples should have a lower PEG content compared to the poor wood samples (Mortensen et al., PEG state and distribution in the Vasa Warship, in preparation). The average formic acid content of these was 0.017% and 0.043%, respectively. Comparisons of dried/PEG-treated wood and low-PEG wood/high-PEG wood both indicate that the formic acid content is linked to the presence of PEG. As the Vasa does not have an unusually high formic acid content in comparison to the other three boats, in which a sulfuric acid problem has not been identified, there is no reason to believe that a solvolysis reaction is occurring in the Vasa wood. Therefore, the degradation reaction previously demonstrated (Glastrup 1996) could be the reason for the increased formic acid.

Our results show that the formic acid content is probably not dependent on the age of the wood. We did not find any formic acid in fresh wood (although the minimum detection level is 0.047%, see Figure 2a), whereas the non-conserved archaeological wood from the Bremen Cog and the Danish Viking Ships used as standards showed a low concentration (Av. 0.022%) of formic acid. This may reflect degradation/hydrolysis of the wood.

In fresh oak wood, acetic acid is present in concentrations between 0.4% and 7%, depending on age, heat treatment, sap- or heartwood, etc. (Packman 1960; Arni et al. 1965; Balaban and Ucar 2003; Sundqvist 2004). Our results show that the acid content declines over time, but that 1000-year-old wood still contains measurable amounts. This is in contrast to previous analysis (Westermark et al. 2005), which found only trace amounts in 350-year-old wood. There are no indications, although the sample numbers were low, that the acetic acid content depends on the PEG content, and it is therefore probable that the acetic acid originates from the wood.

From a conservation and curatorial point of view, the presence of formic and acetic acids is a point of concern. There is not much doubt that even a low concentration of these acids in wood leads to corresponding concentrations in the surrounding air. Huge potential for corrosion of metallic and calcareous objects exists in the areas surrounding PEG-treated wooden artefacts, not only as a result of the formic and acetic acids originating from the wood, but also from the PEG that has been used for the conservation.

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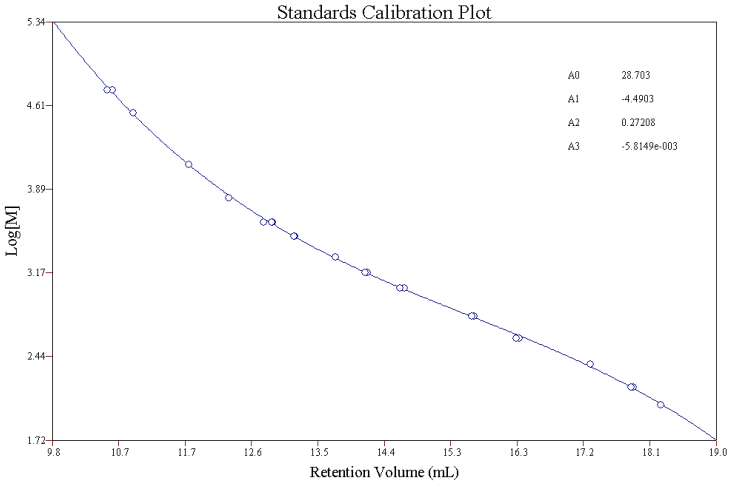
Appendix 4

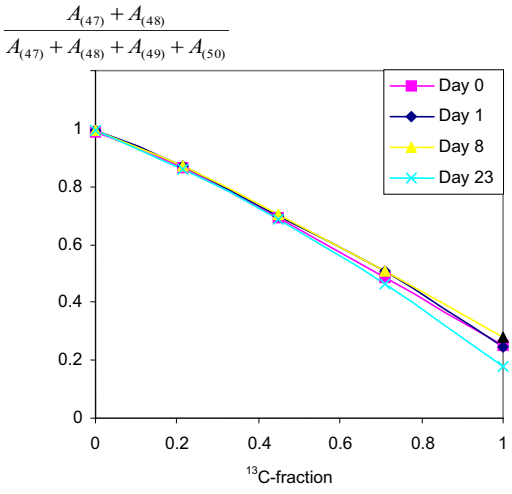
Mass calibration



Size Exclusion Chromatography (SEC) mass calibration curve for polyethylene glycol PEG. The logarithm (log) to the molecular weights of the PEG standards that were tested on the chosen set of columns is plotted versus retention expressed as the volume of eluent in ml.

Appendix 5

Stability over time



Five solutions containing mixtures of $H^{12}COOH$ and $D^{13}COOH$ (X-axis) were measured four times by SPME GC-MS over 23 days. Integrals of (*m*/*z* 47 + *m*/*z* 48)/(*m*/*z* 47 + *m*/*z* 48 + *m*/*z* 49 + *m*/*z* 50) are plotted as a function of the theoretical ^{13}C-fraction. It is seen that the curves change very little over this time period.

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